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**BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant: Bosch et al.
Title: ***DRY POWDER AEROSOLS OF NANOPARTICULATE DRUGS***
Appl. No.: 09/190,138
Filing Date: November 12, 1998
Examiner: James Henry Alstrum-Acevedo
Art Unit: 1615

THIRD TRANSMITTAL OF AMENDED BRIEF ON APPEAL

Mail Stop APPEAL BRIEF-PATENTS
Commissioner for Patents
PO Box 1450
Alexandria, Virginia 22313-1450

Sir:

Transmitted herewith is an appeal brief in the above-identified application:

Submitted herewith in connection with the above application are the following:

- ☒ [X] Appellant's Third Amended Brief on Appeal.
- ☒ [X] Evidence Appendix (containing 10 references).

- [] Please charge Deposit Account No. 19-0741 in the amount of \$00.00. A duplicate copy of this transmittal is enclosed.
- [X] The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of papers submitted herewith, applicant hereby petitions for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 19-0741.

Please direct all correspondence to the undersigned attorney or agent at the address indicated below.

Respectfully submitted,

Date October 6, 2006

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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES**

Applicant: Bosch et al.

Title: ***DRY POWDER AEROSOLS OF NANOPARTICULATE DRUGS***

Appl. No.: 09/190,138

Filing Date: 11/12/1998

Examiner: James Henry Alstrum-Acevedo

Art Unit: 1615

APPELLANT'S THIRD AMENDED BRIEF ON APPEAL UNDER 37 C.F.R. § 41.37

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Under the provisions of 37 C.F.R. § 41.37 and in response to the Notification of Non-Compliant Appeal Brief mailed on September 27, 2006 ("Notification"), Appellants submit this Third Amended Appeal Brief. The Notification stated that Appellants' previously amended appeal brief, filed on March 17, 2006, was non-compliant because the "claimed invention is not mapped to the independent claims on appeal." The present brief explains the subject matter that is claimed in each of the independent claims on appeal, referring to the specification by page and line number.

The Authorization is hereby given to charge any deficiency (or credit any balance) to the undersigned deposit account 19-0741 if any fees are due.

I. REAL PARTY IN INTEREST

The real party in interest is ELAN PHARMA INTERNATIONAL, LTD. The inventors assigned their right, title, and interest in this application to NANOSYSTEMS on January 6 and 11, 1999. This assignment was recorded in the U.S. Patent and Trademark Office at Reel 009861, Frame 0227.



NANOSYSTEMS subsequently assigned its right, title, and interest in this application to ELAN PHARMA INTERNATIONAL, LTD. This assignment was recorded in the U.S. Patent and Trademark Office at Reel 011079, Frame 0301.

II. RELATED APPEALS AND INTERFERENCES

One of the grounds for deeming Appellant's Brief on Appeal non-compliant was that Appellants allegedly failed to identify all related appeals and interferences. Appellants believe that there are no related appeals or interferences. They acknowledge, however, their appeal of a final rejection of claims in U.S. Patent Application No. 09/577,489 (the "'489 application") (prosecution in this case has now been re-opened by the Examiner). However, the '489 application is not legally related to the present application. Additionally, the claims of the '489 application are directed to liquid droplet aerosols, in contrast to the presently claimed dry powder aerosols. Thus, the subject matter underlying the appeal in the '489 application is unrelated to the presently claimed invention.

There are no other appeals or interferences related to the present application.

III. STATUS OF CLAIMS

Pending claims: 11-36, 40-45, 47-49, and 51-121.

Rejected claims: 11-36, 40-45, 47-49, and 51-121.

Appealed claims: 11-36, 40-45, 47-49, and 51-121.

IV. STATUS OF AMENDMENTS

An after-final response, filed on May 14, 2004, sought to amend independent claims 11, 23, 35, 40, and 42-44. In light of the Advisory Action dated July 22, 2004, and according to the Examiner's Answer entered on May 26, 2006, Appellants understand that the amendments have been

entered for purposes of the present appeal. Consequently, the appended claims and their status identifiers reflect the entry of the amendments.

V. SUMMARY OF CLAIMED SUBJECT MATTER

The claimed invention is directed to dry powder aerosol compositions of nanoparticulate crystalline drugs for pulmonary and nasal delivery and to methods of making and using the compositions. *See* specification at page 7, lines 24-25 and page 16, lines 11-12. The claimed compositions comprise spherically-shaped aggregates of crystalline drug nanoparticles having a surface modifier adsorbed to the surface thereof. *Id.* at page 17, lines 6-7; page 20, lines 23; and page 33, line 16. The drug particles must be crystalline, *id.* at page 24, lines 26-28, and exhibit an effective average particle size of less than about 1 micron. *Id.* at page 7, lines 5-6 and page 13, lines 2-8. The diameters of the spherically-shaped aggregates are equal to or less than about 100 microns. *Id.* at page 17, lines 10-18. Finally, the aggregates must yield back the nanoparticulate drug dispersions upon reconstitution in an aqueous medium. *Id.* at page 8, line 28; page 9, line 17; page 33, lines 19-22; and page 34, lines 28-30.

Now having generally identified textual support for the claimed invention, Appellants present the following chart that identifies precisely what passages of the specification describe the subject matter as claimed in each independent claim. 37 C.F.R. § 41.67(c)(1)(v).

| Independent Claim | Claimed Subject Matter | Support in the Specification |
|-------------------|--|---|
| 11 | dry powder aerosol compositions, formed from spray-drying, of nanoparticulate crystalline drugs for pulmonary and nasal delivery | page 7, lines 24-25; page 8, line 9 to page 9, line 2; and page 16, lines 11-12 |
| | spherically-shaped aggregates of crystalline drug nanoparticles having a surface modifier adsorbed to the surface thereof | page 17, lines 6-7; page 20, lines 23; and page 33, line 16 |
| | aggregates are less than or equal to about 100 microns in diameter | page 4, lines 11-12; page 13, lines 27-28; and page 21, line 4 |
| | drug particles must be crystalline | page 24, lines 26-28 |

| Independent Claim | Claimed Subject Matter | Support in the Specification |
|-------------------|--|--|
| | effective average particle size of less than about 1 micron | page 7, lines 5-6 and page 13, lines 2-8 |
| | aggregates must yield back the nanoparticulate drug dispersions upon reconstitution in an aqueous medium | page 8, line 28; page 9, line 17; page 33, lines 19-22; and page 34, lines 28-30 |
| 23 | dry powder aerosol compositions, formed from spray-drying, of nanoparticulate crystalline drugs for pulmonary and nasal delivery | page 7, lines 24-25; page 9, lines 4-20; and page 16, lines 11-12 |
| | aggregates of crystalline drug nanoparticles having a surface modifier adsorbed to the surface thereof | page 17, lines 6-7; page 20, lines 23; and page 33, line 16 |
| | aggregates are less than or equal to about 100 microns in diameter | page 4, lines 11-12; page 13, lines 27-28; and page 21, line 4 |
| | drug particles must be crystalline | page 24, lines 26-28 |
| | effective average particle size of less than about 1 micron | page 7, lines 5-6 and page 13, lines 2-8 |
| | aggregates must yield back the nanoparticulate drug dispersions upon reconstitution in an aqueous medium | page 8, line 28; page 9, line 17; page 33, lines 19-22; and page 34, lines 28-30 |
| | | |
| 35 | dry powder aerosol compositions for use in propellant-based pMDI | page 9, line 22 to page 10, line 5; page 17, line 23 to page 18, line 3 |
| | spherically-shaped aggregates of poorly soluble crystalline drug particles | page 17, lines 4-19; page 24, lines 26-28 and 30 |
| | aggregates are less than or equal to about 100 microns in diameter | page 4, lines 11-12; page 13, lines 27-28; and page 21, line 4 |
| | aggregates must yield back the nanoparticulate drug dispersions upon reconstitution in an aqueous medium | page 8, line 28; page 9, line 17; page 33, lines 19-22; and page 34, lines 28-30 |
| | nanoparticles having a surface modifier adsorbed to the surface thereof | page 17, lines 6-7; page 20, lines 23; and page 33, line 16 |
| | effective average particle size of less than about 1 micron | page 7, lines 5-6 and page 13, lines 2-8 |
| | non-aqueous propellant | page 10, lines 14-28 |
| 40 | Method of making dry powder nanoparticulate drug composition | page 10, lines 7-15; abd page 18, lines 5-15 |

| Independent Claim | Claimed Subject Matter | Support in the Specification |
|-------------------|--|--|
| | Forming aqueous nanoparticulate dispersion of a poorly soluble drug | Page 8, line 10-12 |
| | Drug solubility in at least one liquid dispersion medium is less than about 10 mg/mL | Page 25, lines 1-3 |
| | Drug is crystalline | Page 24, lines 24-27 |
| | nanoparticles have a surface modifier adsorbed to the surface thereof | page 17, lines 6-7; page 20, lines 23; and page 33, line 16 |
| | effective average particle size of less than about 1 micron | page 7, lines 5-6 and page 13, lines 2-8 |
| | Spray-drying nanoparticulate dispersion to form dry powder of spherically shaped aggregates | Page 8, line 10-12 |
| | aggregates are less than or equal to about 100 microns in diameter | page 4, lines 11-12; page 13, lines 27-28; and page 21, line 4 |
| | aggregates must yield back the nanoparticulate drug dispersions upon reconstitution in an aqueous medium | page 8, line 28; page 9, line 17; page 33, lines 19-22; and page 34, lines 28-30 |
| 42 | Method of making dry powder nanoparticulate drug aerosol formulation | Page 10, lines 11-12 and 16-21 |
| | Milling under non-pressurized conditions in a non-aqueous medium having a high boiling point a dispersion | Page 10, lines 16-21; and page 19, lines 15-16 |
| | dispersion comprises a poorly soluble drug | Page 8, line 10-12 |
| | Drug solubility in at least one liquid dispersion medium is less than about 10 mg/mL | Page 25, lines 1-3 |
| | Drug is crystalline | Page 24, lines 24-27 |
| | nanoparticles have a surface modifier adsorbed to the surface thereof | page 17, lines 6-7; page 20, lines 23; and page 33, line 16 |
| | effective average particle size of less than about 1 micron | page 7, lines 5-6 and page 13, lines 2-8 |
| | Evaporating non-aqueous medium to obtain a dry powder of spherically shaped aggregates of drug and surface modifier particle | Page 19, lines 20-21 |

| Independent Claim | Claimed Subject Matter | Support in the Specification |
|-------------------|---|--|
| | aggregates are less than or equal to about 100 microns in diameter | page 4, lines 11-12; page 13, lines 27-28; and page 21, line 4 |
| | aggregates must yield back the nanoparticulate drug dispersions upon reconstitution in an aqueous medium | page 8, line 28; page 9, line 17; page 33, lines 19-22; and page 34, lines 28-30 |
| | formulating the dry powder spherically shaped aggregates into an aerosol composition | Page 19, lines 21-22 |
| 43 | Method of making an aerosol composition comprising milling under pressurized conditions | Page 20, lines 1-9 |
| | dispersion comprises a poorly soluble drug | Page 8, line 10-12 |
| | Drug solubility in at least one liquid dispersion medium is less than about 10 mg/mL | Page 25, lines 1-3 |
| | Drug is crystalline | Page 24, lines 24-27 |
| | nanoparticles have a surface modifier adsorbed to the surface thereof | page 17, lines 6-7; page 20, lines 23; and page 33, line 16 |
| | effective average particle size of less than about 1 micron | page 7, lines 5-6 and page 13, lines 2-8 |
| | Evaporating non-aqueous medium to obtain a dry powder of spherically shaped aggregates of drug and surface modifier particle | Page 19, lines 20-21 |
| | aggregates are less than or equal to about 100 microns in diameter | page 4, lines 11-12; page 13, lines 27-28; and page 21, line 4 |
| | aggregates must yield back the nanoparticulate drug dispersions upon reconstitution in an aqueous medium | page 8, line 28; page 9, line 17; page 33, lines 19-22; and page 34, lines 28-30 |
| 44 | Method of making dry powder nanoparticulate drug composition comprising forming an aqueous nanoparticulate dispersions of poorly soluble drug | Page 18, lines 19-25 |
| | dispersion comprises a poorly soluble drug | Page 8, line 10-12 |

| Independent Claim | Claimed Subject Matter | Support in the Specification |
|-------------------|--|--|
| | Drug solubility in at least one liquid dispersion medium is less than about 10 mg/mL | Page 25, lines 1-3 |
| | Drug is crystalline | Page 24, lines 24-27 |
| | nanoparticles have a surface modifier adsorbed to the surface thereof | page 17, lines 6-7; page 20, lines 23; and page 33, line 16 |
| | effective average particle size of less than about 1 micron | page 7, lines 5-6 and page 13, lines 2-8 |
| | Freeze-drying the nanoparticulate dispersion to form a dry powder of spherically shaped aggregates | Page 22, lines 11-19 |
| | aggregates are less than or equal to about 100 microns in diameter | page 4, lines 11-12; page 13, lines 27-28; and page 21, line 4 |
| | aggregates must yield back the nanoparticulate drug dispersions upon reconstitution in an aqueous medium | page 8, line 28; page 9, line 17; page 33, lines 19-22; and page 34, lines 28-30 |

Appellants' discovery is a substantial advance over conventional micronized drug formulations that are intended for delivery to the pulmonary and nasal regions. The nanoparticulate drug particle size facilitates the drug particles' fitting into a wide aggregate size range. Consequently, aggregate particles measuring less than about 2 microns up to about 100 microns uniformly contain the same concentration of nanoparticulate drug particles, by contrast to conventional micronized formulations, and thereby allow more effective and efficient drug delivery to a desired region. *See* specification at page 23, line 26 to page 24, line 6. Moreover, the present invention results in more favorable drug delivery profiles relative to micronized drugs on account of the nanoparticulate drug size allowing more concentrated unit doses. *Id.* at page 23, lines 17-22. Additionally, a number of advantageous bulk properties result from the instant compositions. For example, the present compositions can be nebulized ultrasonically whereas micronized drug dispersions cannot. *Id.* at page 23, lines 23-25. By contrast to micronized drugs, the present invention also enables very rapid drug delivery to pulmonary and nasal surfaces. *Id.* at page 24, lines 7-10 and 15-18.

VI. GROUND OF REJECTION TO BE REVIEWED ON APPEAL

Four issues remain after the issuance of the Final Office Action dated January 14, 2004:

- A. Whether claims 11-34, 40, 41, 44, 45, 47, 48, 51-62, 69-96, and 111-119 are unpatentable under 35 U.S.C. § 103(a) over U.S. Pat. No. 5,985,309 to Edwards et al. (“Edwards”).
- B. Whether claims 11-34, 40- 45, 47, 48, 51-62, 65-96, and 97-119 are unpatentable under 35 U.S.C. § 103(a) over Edwards in view of U.S. Pat. No. 5,145,684 et al. to Liversidge (“Liversidge”).
- C. Whether claims 35, 36, 49, 63, and 64 are unpatentable under 35 U.S.C. § 103(a) over Edwards in view of U.S. Pat. No. 5,202,110 to Dalby et al. (“Dalby”).
- D. Whether claims 120 and 121 are unpatentable under 35 U.S.C. § 103(a) over Edwards in view of Goodman & Gilman’s, The Pharmacological Basis of Therapeutics, Ninth edition, page 666 (McGraw-Hill, 1996) (“Goodman”).

VII. ARGUMENT

The claimed invention is patentable over the cited prior art because the PTO has not established a *prima facie* case of obviousness.

To establish a *prima facie* case of obviousness, three basic criteria must be met. First, the prior art references (or references when combined) *must teach or suggest all of the claim limitations*. Second, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Finally, there must be a reasonable expectation of success.

See MPEP § 2143 (Rev. 2, May 2004) (emphasis added).

Specifically, *none* of the cited references, alone or in combination with each other, teach or suggest the following elements that are required by each of Appellants’ claims: (1) spherically-shaped aggregates of drug nanoparticles having a surface modifier adsorbed to the surface thereof, (2) the drug nanoparticles are crystalline and (3) have an effective average particle size of less than

about 1 micron, and (4) the spherically-shaped aggregates must yield back nanoparticulate drug dispersions upon reconstitution in an aqueous medium. Furthermore, the PTO has not properly established a motivation to modify Edwards alone or by any of the cited secondary references.

A. Issue 1: Edwards

Edwards does not teach or suggest each limitation of the claimed invention, nor would a person of ordinary skill in the art have been motivated to modify the reference in a manner such as to arrive at the claimed invention. Appellants note in this regard that each of the outstanding rejections turns wholly or primarily upon whether Edwards pertains.

1. The PTO Applied an Improper Standard in Determining Whether Edwards Teaches or Suggests a *Nanoparticulate Drug*

Appellants have argued at length throughout the prosecution of the present application that the *microparticulate* drug compositions disclosed by Edwards simply do not amount to or suggest the presently recited *nanoparticulate* drug dispersion. Thus Edwards discloses particles that comprise a therapeutic agent and a surfactant for administration to the respiratory tract *via* an aerosol formulation. *See* Edwards at col. 3, lines 1-3 and col. 5, lines 29-33. Specifically, the disclosed aerosol particles have “a mean diameter of between approximately 5 μm and 30 μm .” Edwards at col. 6, lines 1-7. Additionally, Edwards discloses a number of processes for making particles that satisfy these size requirements. *See, e.g.*, Edwards at col. 8., lines 5-29. Consequently, none of the disclosed particles nor the processes for making them remotely suggests that the particles comprise *nanoparticulate* drug.

a. Edwards Does Not Teach or Suggest Drug *Nanoparticles*

Despite the paucity of evidence that the compositions of Edwards somehow implicate drug nanoparticles, the PTO has persistently buttressed a majority of its obviousness determination by a two-pronged platform that enjoys not even the flimsiest factual or legal support. Not once during the pendency of this application has the PTO unequivocally established that Edwards teaches drug

nanoparticles, much less those that form spherically-shaped aggregates as required by Appellants claims. Notably, the PTO recognized that “Edwards *et al.* do not specifically state that 50% of the [disclosed] particles have a geometric particle size of less than about 1 μm [as claimed].” Office Action, dated February 5, 2001, at page 5. Nonetheless, as one pillar of the PTO’s platform is the “examiner’s position that Edwards disclosed that nanosize drug particles in aerosol form were delivered to the alveoli of the lung col 3 [*sic*; “lung, col. 3], lin [*sic*] 330-35 [*sic*; “33-35”] and col 5 [*sic*], lin [*sic*] 30-40). [*sic*]”

Edwards simply teaches nothing of the sort. *See* Appellants’ Response, dated May 14, 2004, at pages 23-24. Edwards teaches that “[a]dministration by aerolization permits deep lung delivery of *relatively large diameter* therapeutic aerosols, for example, greater than 5 μm in mean diameter.” Edwards at col. 5, lines 31-34 (emphasis supplied). Thus, Edwards flatly refutes the PTO’s contention that the reference teaches nanoparticulate drug on account of drug particles reaching the deep lung.

As to the second pillar of the PTO’s platform, the PTO invoked an incorrect legal standard by persistently requiring Appellants to first establish the recited nanoparticulate drug size as a critical feature of the invention before patentability over Edwards would be acknowledged. It is black letter law that “[t]he examiner bears the burden of establishing a *prima facie* case of obviousness.” *In re Deuel*, 51 F. 3d 1552, 1557 (Fed. Cir. 1995) (citing *In re Rijckaert*, 9 F. 3d 1531, 1532 (Fed. Cir. 1993); *In re Oetiker*, 977 F. 2d 1443, 1445 (Fed. Cir. 1992)). “*Only if* this burden is met does the burden of coming forward with rebuttal argument or evidence shift to the applicant.” *Rijckaert*, 9 F. 3d at 1532 (emphasis supplied). In this analysis, “[a]ll words in a claim must be considered in judging the patentability of that claim against the prior art.” *In re Wilson*, 424 F. 2d 1382, 1385 (CCPA 1970). In particular, “[o]bviousness requires a suggestion of all elements in a claim.” *In re Royka*, 490 F. 2d 981 (CCPA 1974). The PTO therefore is obligated to consider that Appellants’

claimed composition comprises nanoparticulate drug particles and to bear the initial burden of determining whether Edwards teaches or suggests this feature of Appellants' invention.

By contrast to the principles stated above, the PTO improperly "granted [no] patentable weight" to the "merely superfluous" nanoparticulate size limitation, *see* Office Action dated August 21, 2000 at page 5, and consequently adopted without explanation the notion that "the compositions of the instant claims and those of [Edwards] do not appear to be different." Office Action dated August 27, 2001 at page 5. In this context, the PTO has now maintained the present rejection because Appellants "did not present factual evidence showing criticality of size of the nanoparticles of drug in the instant claims that is different than the size of particles of drug in the [cited prior art]; *specifically Edwards*." Advisory Action dated July 22, 2004 at page 2 (emphasis supplied).

Appellants discussed at length above and throughout prosecution that Edwards does not teach or suggest nanoparticulate drug particles as required by each of Appellants' claims. Under no principle does the PTO have the authority to simply ignore or characterize as "merely superfluous" this feature of Appellants' invention when establishing a *prima facie* case of obviousness. *See Wilson*, 424 F. 2d at 1385 (CCPA 1970). Since the PTO has failed to show that Edwards teaches or suggests this feature of Appellants' invention, the PTO has not established the requisite *prima facie* case of obviousness. In any event, Appellants are under no obligation, by contrast to the PTO's reasoning, to proffer evidence or argument as to why their invention is patentable over Edwards.

b. Edwards Teaches Away From Appellants' Aggregates Comprising Nanoparticulate Drug Particles

The PTO has not established a *prima facie* case of obviousness for the additional reason that a person of ordinary skill in the art would not have been motivated by Edwards to make the recited nanoparticulate drug particles. "In determining the propriety of the Patent Office case for obviousness in the first instance, it is necessary to ascertain whether or not the reference teachings would appear to be sufficient for one of ordinary skill in the relevant art having the reference before

him to make the proposed substitution, combination, or other modification.” *In re Linter*, 458 F. 2d 1013, 1016 (CCPA 1972). In the present case, there is no such “proposed substitution, combination, or other modification” embodied in the PTO’s conclusory opinion that the “compositions of the instant claims and those of [Edwards] do not appear to be different.” Office Action dated August 27, 2001 at page 5. The PTO nonetheless shoulders the burden of establishing that the person of ordinary skill would have motivated to modify the *microparticulate* composition disclosed by Edwards to arrive at Appellants’ claimed compositions that comprise *nanoparticulate* drug particle. There is no motivation whatsoever.

Edwards teaches away from Appellants’ invention because the reference strongly suggests that nanoparticles would be ineffective for delivery of a drug to the pulmonary system. “[R]eferences that teach away cannot serve to create a prima facie case of obviousness.” *McGinley v. Franklin Sports, Inc.*, 262 F. 3d 1339, 1354 (Fed. Cir. 2001) (citing *In re Gurley*, 27 F. 3d 551, 553 (Fed. Cir. 1994)). Here, Edwards teaches that larger drug particles, *e.g.*, those having a mean diameter between 5 μ m and 30 μ m, are used to provide maximum deposition of the drug within the lungs. *See* Edwards at col. 5, lines 10-15. Moreover, the larger particles of Edwards avoid the fate of “very small particles of less than five microns in diameter, preferably between one and three microns in diameter, which are then subject to phagocytosis.” Edwards at col. 5, lines 16-18. Edwards thus teaches that inhalation of larger, not smaller, drug particles are necessary for delivery of the drug to the lungs to avoid the risk of phagocytosis. Consequently, a person of ordinary skill in the art would have been counseled by Edwards against making smaller drug particles for their delivery to the lung. The person therefore would not have been motivated to make Appellants’ claimed invention comprising nanoparticulate drug particles.

2. Edwards Does Not Teach or Suggest *Crystalline* Drug Nanoparticles

Even if Edwards suggested the recited drug nanoparticles, a proposition that Appellants' do not endorse for the foregoing reasons, the reference does not teach or suggest that the drug must be crystalline as required by each of Appellants' claims. Edwards teaches several methods of manufacturing the disclosed drug microparticles, including emulsion solvent evaporation and spray drying, each of which result in *amorphous* drug substances. *See* Appellants' Response dated July 11, 2002 at page 6. *See also* Edwards at col. 8, lines 7-10; col. 13, lines 49 to col. 14, line 19 (Example 1; evaporation); col. 14, lines 21 to 49 (Example 2; spray-drying).

By contrast to the amorphous particles of Edwards, Appellants' claimed invention comprises crystalline drug nanoparticles. In light of this difference, the PTO has not demonstrated that a person of ordinary skill in the art would have been motivated to modify the compositions of Edwards to arrive at dry powder aggregates comprising nanoparticulate *crystalline* drug. Absent such a motivation, a *prima facie* case of obviousness does not exist. *Linter*, 458 F. 2d at 1016 (CCPA 1972).

3. Edwards Does not Teach or Suggest Spherically Shaped Aggregates

Appellants' invention is further patentable over Edwards because the reference does not teach or suggest aggregates that are spherically-shaped as required by each of Appellant's claims. In particular, the disclosed microparticles are "rough (non-smooth) [and] non-spherical . . ." Edwards at col. 9, lines 15-17.

Moreover, as discussed at length by Appellants, the PTO has indicated no motivation whatsoever for one of ordinary skill in the art to modify the rough particles of Edwards to obtain the smooth particles of the claimed invention. *See* Appellants' Response dated May 14, 2004 at page 22. In this regard, Edwards teaches away from Appellants' invention because the reference emphasizes that the aerodynamic lightness of the disclosed particles is achieved in part by creating irregular surface structures on the particles. *See* Edwards at col. 5, lines 64-67. It is therefore irrelevant, in

contrast to the PTO's reasoning, that "the prior art as known and expressed in Edwards teaches smooth and spherical microparticle drug for inhalation . . ." Office Action dated January 14, 2004. What is relevant is that Edwards itself does not teach or suggest this feature of Appellants' invention.

4. Edwards Does Not Teach or Suggest Aggregates That Yield Nanoparticulate Drug Dispersions

The PTO has not rebutted Appellants' arguments that Edwards does not teach or suggest that the claimed aggregates of nanoparticulate drug return to nanoparticulate drug particle dispersions upon reconstitution in an aqueous liquid medium. *See* Appellants' Response dated May 14, 2004 at page 25. "Obviousness requires a suggestion of all elements in a claim." *In re Royka*, 490 F. 2d 981 (CCPA 1974). The PTO is thus required in establishing its *prima facie* case of obviousness to show that Edwards suggests this feature of Appellants' invention. The PTO did not.

Applicants understand the PTO in this context to characterize the reference as teaching the variance of a number of parameters that would facilitate the disclosed particles in reaching selected regions of the respiratory tract. *See* Office Action dated January 14, 2004 at page 3 (citing Edwards at col. 10, lines 45-55). This is irrelevant: the reference still does not teach or suggest that aggregates as claimed are reconstituted into nanoparticulate drug particle dispersions. In fact, Edwards teaches instead that the disclosed large and aerodynamically light drug particles simply "undergo slow degradation and drug release." Edwards at col. 10, lines 37-38.

5. Conclusion

Because Edwards does not teach or suggest all of the limitations of Appellants' claims, and because the PTO has failed to identify a suggestion or motivation to modify Edwards to arrive at the

claimed invention, the PTO has not established a *prima facie* case of obviousness. Appellants therefore respectfully request the Board to reconsider and reverse this ground for rejection.

B. Issue 2: Edwards in View of Liversidge

Edwards in combination with Liversidge does not obviate the claimed invention because Liversidge remedies none of the deficiencies of Edwards discussed above and because the references militate against making the claimed aerosol composition. Appellants' discussion of Edwards, *supra*, follows lengthy discourse during prosecution to demonstrate that Liversidge does not cure the deficiencies of Edwards. Specifically, Liversidge is directed to a composition of a "crystalline drug substance having a surface modifier adsorbed on the surface thereof . . . to maintain an effective average particle size of less than about 400 nm." Liversidge at col. 2, lines 38-43. The nanoparticulate compositions of Liversidge are disclosed as being useful in oral, parenteral, and intravenous administration applications. *See* Liversidge at col. 8, lines 10-13. By contrast, Edwards discloses "aerodynamically light particles generally hav[ing] a mean diameter between 5 μm and 30 μm ", Edwards at col. 4, lines 1-4, for "delivery of a therapeutic agent to the airways of the alveolar region of the lung." *Id.* at col. 4, lines 19-20. The references thus embody (1) non-obvious variants of drug compositions and (2) non-overlapping routes of administration. Neither reference therefore suggests the claimed aggregate of nanoparticulate drug dispersions nor does the PTO demonstrate how one of ordinary skill in the art would modify the light particles of Edwards with the comparatively dense crystalline particles of Liversidge to arrive at the claimed invention.

Edwards teaches away from the proposed combination because the reference teaches that small drug particles are not useful in the context of pulmonary drug delivery. "If references taken in combination would produce a 'seemingly inoperative device,' . . . such references teach away from the combination and thus cannot serve as predicates for a *prima facie* case of obviousness." *See McGinley v. Franklin Sports, Inc.*, 262 F. 3d 1339, 1354 (Fed. Cir. 2001) (quoting *In re Spinnoble*,

405 F. 2d 578, 587 (CCPA 1969)). As noted above, Edwards is directed to large drug particles, *i.e.*, 5 μm to 30 μm , to avoid the fate of “very small particles of less than five microns in diameter, preferably between one and three microns in diameter, which are then subject to phagocytosis.” Edwards at col. 5, lines 16-18. A person of ordinary skill in the art would therefore have been dissuaded by Edwards from using the nanoparticulate compositions of Liversidge in a modification of Edwards to arrive at the claimed dry powder aerosol.

The PTO propounded two theories in fortifying the ground for maintaining this rejection. The facts in this case support neither theory. First, the PTO pronounced that “the disclosures in Liversidge [*sic*, “Liversidge”] . . . are in the same field of endeavor as that in Edwards – nanosize drug particles that are surface modified in liquid dispersion.” Office Action dated January 14, 2004 at page 4. Appellants have pointed out that Edwards and Liversidge are *not* both directed to nanoparticulate drug particles. *See* Appellants’ Response dated May 14, 2004 at page 27. Specifically, as discussed above, nowhere does Edwards teach or suggest drug nanoparticles.

Second, the PTO styled Liversidge as “address[ing] similar problems that were raised in Edwards concerning nanosize drug delivery formulations through the respiratory tract . . .” Even a cursory inspection of Liversidge indicates that this is not so. Thus Appellants’ emphasize here, *supra*, as before that Liversidge teaches “that the pharmaceutical compositions of this invention will be particularly useful in oral and parenteral, including intravenous, administration applications.” Liversidge at col. 8, lines 10-13. Liversidge simply does not address the administration of “nanosize drug delivery formulations through the respiratory tract.” Office Action dated January 14, 2004 at page 4.

The proposed combination of Edwards and Liversidge therefore does not teach or suggest all of the limitations of Appellants’ claims, specifically the recited aggregates comprising nanoparticulate drug dispersions. Moreover, Edwards teaches away from the combination because the reference specifically teaches the inoperability of the aerosol delivery of small drug particles. For

these reasons, the combination of references does not obviate the claimed invention. Consequently, Appellants respectfully request the Board to reconsider and reverse this ground for rejection.

C. Issue 3: Edwards in View of Dalby

The combination of Edwards and Dalby does not obviate the claimed invention because Edwards is not a competent reference and because Dalby fails to remedy the deficiencies of Edwards. The PTO relies upon Edwards here “for all that it teaches as stated previously”, Office Action dated December 24, 2004 at page 4, and in particular for the reference’s alleged disclosure of “spherical, nanosize aerosol particles of drug . . .” Office Action dated January 14, 2004 at page 4. Additionally, the PTO cites Dalby “for teaching propellant metered dose inhalers where the propellant is a ‘non-CFC’ propellant.” *See* Office Action dated December 24, 2004 at page 4. *See also* Office Action dated January 14, 2004 at page 4.

For all of the foregoing reasons, Edwards does not teach or suggest the claimed invention. Specifically, nowhere does Edwards disclose the cited “spherical, nanosize aerosol particles of drug . . .” By contrast to the PTO’s characterization of the reference, Edwards teaches aerosol particles that have “a mean diameter of between approximately 5 μm and 30 μm ”, Edwards at col. 6, lines 1-7, that are rough (non-smooth) [and] non-spherical . . .” *Id.* at col. 9, lines 15-17. For at least these reasons, this ground for rejection is moot to the extent that the PTO relies upon Edwards “for all that it teaches . . .” Consequently, this ground for rejection turns upon whether the PTO’s reliance upon Dalby is well-founded. It is not.

Dalby, as with Edwards, does not teach or suggest aggregates of crystalline, nanoparticulate drug particles as required by each of Appellants’ claims. Dalby is directed to formulations of beclomethasone dipropionate for aerosol delivery *via* a metered dose inhaler. *See* Dalby at col. 2, lines 22-26. In particular, the reference teaches that “the micronized drug . . . should be easily dispersible in the propellant or propellant blend with the aid of a surfactant, or completely dissolve.”

Id. at col. 2, lines 36-38. Dalby therefore does not teach or suggest that the disclosed drug particles are or should be crystalline, much less that the particles form spherically-shaped aggregates measuring less than or equal to about 100 microns in diameter as claimed.

The references do not support the PTO's *prima facie* case of obviousness because Edwards, alone or in combination with Dalby, does not teach or suggest each and every element of the claimed invention. Accordingly, Appellants respectfully request the Board to reconsider and reverse this ground for rejection.

D. Issue 4: Edwards in View of Goodman

The combination of Edwards and Goodman also fails to teach or suggest the claimed invention because Goodman does not remedy those deficiencies of Edwards discussed above. In this context, the PTO relied upon Goodman simply to the extent that the reference "teaches . . . beclomethasone dipropionate . . . [as a] steroid administered for asthma in aerosol formulations." Office Action dated December 24, 2002 at page 6.

While Appellants affirm this characterization of Goodman, the PTO's commentary nonetheless falls short of establishing a *prima facie* case of obviousness based upon the proposed combination. Thus, Goodman does not address the requisite (1) spherically-shaped aggregates of drug nanoparticles having a surface modifier adsorbed to the surface thereof, (2) drug nanoparticles that are crystalline and (3) that have an effective average particle size of less than about 1 micron, and (4) spherically-shaped aggregates that must yield back nanoparticulate drug dispersions upon reconstitution in an aqueous medium. Because Edwards also does not teach or suggest any of these claimed features of Appellants' invention, *supra*, the combination of Edwards and Goodman also fails to do so.

Assuming, *arguendo*, that the references could be combined to satisfy all of the claimed limitations, the PTO's obviousness determination still inadequately addresses the motivation to

combine the references. *See In re Linter*, 458 F.2d 1013, 1016 (CCPA 1972). The PTO asserted in this context that Goodman's disclosure of beclomethasone dipropionate being available in aerosol formulations and therefore useful for treating asthma suggests the use of the drug in the formulations of Edwards. Office Action dated December 24, 2002 at page 6. With nothing more, Goodman merely suggests the selection of beclomethasone dipropionate over or in addition to any other drug for use in aerosol administration. The PTO therefore has not established that Goodman would have suggested to a person of skill in the art to modify Edwards, against the specific teaching of Edwards, to provide for spherically-shaped aggregates of crystalline, submicron-sized drug nanoparticles.

Because Edwards, alone or in combination with Goodman, does not teach or suggest all of the claimed features of Appellants' invention, and because Goodman would not have suggested modifying Edwards to arrive at the claimed invention, the proposed combination does not support a *prima facie* case of obviousness. Accordingly, Appellants respectfully request the Board to reconsider and reverse this ground for rejection.

VIII. CLAIMS APPENDIX

Appellants append hereto the claims on appeal as identified in section III. *Supra*.

IX. EVIDENCE APPENDIX

In further response to the Notice, Appellants additionally append copies of the following evidence that was introduced and entered into the record during prosecution:

1. P. Byron, "Aerosol Formulation, Generation, and Delivery Using Nonmetered Systems," *Respiratory Drug Delivery*, 144-151 (CRC Press, 1989); submitted with Appellants' Response filed on November 27, 2000 and "acknowledged" by the PTO in the Office Action mailed on February 5, 2001.
2. a. U.S. Pat. No. 5,145,684;
b. "Test Method B527-93(2000)e1 Standard Test Method for Determination of Tap Density of Metallic Powders and Compounds," <http://www.astm.org/DATABASE.CART/PAGES/B527.htm> ;

- c. "Autotap and Dual Autotap," <http://www.quantachrome.com/Density/Autotap.htm> ;
- d. "Optimal Tapped Density Tester," <http://optimalcontrol.com/tapped.html> ; and
- e. Edwards et al., "Large Porous Particles for Pulmonary Drug Delivery Science," 276:1868-1871 (June 20, 1997);

all submitted with Appellants' Response filed on May 5, 2001 and entered by the PTO in the Office Action mailed on August 27, 2001.

- 3. a. J. M. Ohrt *et al.*, "Crystal Data (II) for some androstanes," *Acta Cryst.* **19** (1965) 479;
- b. A. L. Thakkar *et al.*, "Crystallographic data for testosterone hydrate and anhydrate," *Acta Cryst.* **B26** (1970) 1184; and
- c. J. M. Ohrt *et al.*, "Crystal Data (II) for some estrone-related compounds," *Acta Cryst.* **17** (1964) 1611; and
- d. J. P. Beale *et al.*, "DL-N-tbutyl-2(4-hydroxy-3-hydroxymethylphenyl)2-hydroxyethylamin, (SAL-butamol, Ah.3365), C₁₃H₂₁NO₃," *Cryst. Struct. Comm.* **1** (1972) 71-74;

all submitted with Appellants' Response filed on July 11, 2002 and entered by the PTO in the Office Action mailed on August 27, 2001.

X. RELATED PROCEEDINGS APPENDIX

None.

XI. CONCLUSION

Edwards, alone or in combination with any of the cited secondary references, does not teach or suggest the claimed dry powder aerosol comprising spherically-shaped aggregates of crystalline, submicron-sized drug nanoparticles. Edwards specifically teaches away from the use of heavier smaller drug particles and, in any event, teaches away from the use of spherically-shaped particles. Since none of the secondary references cure these deficiencies of Edwards, or at least suggest that the compositions of Edwards be modified accordingly, the PTO has not established a *prima facie* case of obviousness. For all of these reasons, Appellants courteously request the Board to reconsider and reverse all grounds for rejection of the claims.

Respectfully submitted,

Date Oct. 6, 2006

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CLAIMS APPENDIX: CLAIMS ON APPEAL

1. – 10. (cancelled).

11. (Previously presented) A dry powder aerosol composition for pulmonary or nasal delivery comprising spherically shaped aggregates ~~of~~ formed from spray-drying aqueous dispersions of nanoparticulate drug particles, wherein:

(a) the aqueous dispersions of nanoparticulate drug particles:

(i) comprise a poorly soluble crystalline drug, wherein by “poorly soluble” it is meant that the drug has a solubility in at least one liquid dispersion medium of less than about 10 mg/ml,

(ii) have an effective average particle size of less than about 1000 nm, meaning at least 50% of the drug particles have a particle size of less than about 1000 nm, and

(iii) have a surface modifier adsorbed on the surface thereof; and

(b) the aggregates of such spray-dried drug particle dispersions are less than or equal to about 100 microns in diameter; and

(c) such aggregates return to nanoparticulate drug particle dispersions upon reconstitution in an aqueous liquid medium.

12. (Original) The aerosol composition of claim 11 further comprising a diluent.

13. (Original) The aerosol composition of claim 12, wherein essentially every diluent particle comprises at least one embedded nanoparticulate drug particle having a surface modifier adhered to the surface of the drug particle.

14. (Original) The aerosol composition of claim 11, wherein the drug is selected from the group consisting of proteins, peptides, elastase inhibitors, analgesics, cystic-fibrosis therapies, asthma

therapies, emphysema therapies, respiratory distress syndrome therapies, chronic bronchitis therapies, chronic obstructive pulmonary disease therapies, organ-transplant rejection therapies, therapies for tuberculosis and other infections of the lung, fungal infection therapies, and respiratory illness therapies associated with acquired immune deficiency syndrome, an oncology drug, an anti-emetic, and a cardiovascular agent.

15. (Previously Presented) The aerosol composition of claim 11, wherein the nanoparticulate drug particles have an effective average particle size of less than about 400 nm.

16. (Original) The aerosol composition of claim 11, wherein the aerosol comprises a concentration of a drug in an amount of from about 0.05 mg/g up to about 900 mg/g.

17. (Original) The aerosol composition of claim 16, wherein the aerosol comprises a concentration of a drug selected from the group consisting of about 10 mg/g or more, about 100 mg/g or more, about 200 mg/g or more, about 400 mg/g or more, about 600 mg/g or more, and about 900 mg/g.

18. (Previously Presented) The aerosol composition of claim 11, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of about 2 to about 10 microns.

19. (Previously Presented) The aerosol composition of claim 18, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of about 2 to about 6 microns.

20. (Previously Presented) The aerosol composition of claim 11, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of less than about 2 microns.

21. (Previously Presented) The aerosol composition of claim 11, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of about 5 to about 100 μm .

22. (Previously Presented) The aerosol composition of claim 21, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of about 30 to about 60 μm .

23. (Previously presented) A dry powder aerosol composition for pulmonary or nasal delivery comprising spherically shaped aggregates formed from freeze-drying aqueous dispersions of nanoparticulate drug particles, wherein:

- (a) the aggregates of such freeze-dried drug particle dispersions are less than or equal to about 100 microns in diameter;
- (b) the aqueous dispersions of nanoparticulate drug particles:
 - (i) comprise a poorly soluble crystalline drug, wherein by "poorly soluble" it is meant that the drug has a solubility in at least one liquid dispersion medium of less than about 10 mg/ml,
 - (ii) have an effective average particle size of less than about 1000 nm, meaning at least 50% of the drug particles have a particle size of less than about 1000 nm, and
 - (iii) have a surface modifier adsorbed on the surface thereof; and
- (c) such aggregates return to nanoparticulate drug particle dispersions upon reconstitution in an aqueous liquid medium.

24. (Original) The aerosol composition of claim 23, further comprising a diluent.

25. (Previously Presented) The aerosol composition of claim 23, wherein the drug is selected from the group consisting of proteins, peptides, elastase inhibitors, analgesics, cystic-fibrosis therapies, asthma therapies, emphysema therapies, respiratory distress syndrome therapies, chronic bronchitis therapies, chronic obstructive pulmonary disease therapies, organ-transplant rejection therapies, therapies for tuberculosis and other infections of the lung, fungal infection therapies, and respiratory

illness therapies associated with acquired immune deficiency syndrome, an oncology drug, an anti-emetic, and a cardiovascular agent.

26. (Previously Presented) The aerosol composition of claim 23, wherein the nanoparticulate drug particles have an effective average particle size of less than about 400 nm.

27. (Original) The aerosol composition of claim 23, wherein the aerosol comprises a concentration of a drug in an amount of from about 0.05 mg/g up to about 900 mg/g.

28. (Original) The aerosol composition of claim 27, wherein the aerosol comprises a concentration of a drug selected from the group consisting of about 10 mg/g or more, about 100 mg/g or more, about 200 mg/g or more, about 400 mg/g or more, about 600 mg/g or more, and about 900 mg/g.

29. (Previously Presented) The aerosol composition of claim 23, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of about 2 to about 10 microns.

30. (Previously Presented) The aerosol composition of claim 29, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of about 2 to about 6 microns.

31. (Previously Presented) The aerosol composition of claim 23, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of less than about 2 microns.

32. (Previously Presented) The aerosol composition of claim 23, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of about 5 to about 100 μm .

33. (Previously Presented) The aerosol composition of claim 32, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of about 30 to about 60 μm .

34. (Original) The aerosol composition of claim 23, further comprising spray-dried nanoparticulate drug powder, wherein the drug of the freeze-dried nanoparticulate drug powder is either the same or different from the drug of the spray-dried nanoparticulate drug powder.

35. (Previously presented) A dry powder nanoparticulate aerosol composition for use in a propellant-based pMDI comprising

(a) spherically shaped aggregates of a nanoparticulate poorly soluble crystalline drug particles, wherein by “poorly soluble” it is meant that the drug has a solubility in at least one liquid dispersion medium of less than about 10 mg/ml, wherein the aggregates are less than or equal to about 100 microns in diameter, wherein such aggregates return to nanoparticulate drug particles upon reconstitution in an aqueous liquid medium, and wherein the drug particles:

(i) have a surface modifier adsorbed on the surface thereof, and

(ii) have an effective average particle size of less than about 1000 nm, meaning at least 50% of the drug particles have a particle size of less than about 1000 nm, and

(b) a non-aqueous propellant.

36. (Original) The aerosol composition of claim 35, wherein the propellant is a non-CFC propellant.

37. – 39. (Cancelled)

40. (Previously presented) A method of making a dry powder nanoparticulate drug composition comprising:

(a) forming an aqueous nanoparticulate dispersion of a poorly soluble drug, wherein:

(i) the dispersion comprises poorly soluble crystalline drug particles and a surface modifier adsorbed on the surface thereof, wherein by “poorly soluble” it is meant that

the drug has a solubility in the liquid dispersion medium of less than about 10 mg/ml,
and

(ii) the drug particles have an effective average particle size of less than about 1000 nm,
meaning at least 50% of the drug particles have a particle size of less than about 1000
nm; and

(b) spray-drying the nanoparticulate dispersion to form a dry powder of spherically shaped
aggregates of the nanoparticulate drug and surface modifier particles, wherein the
aggregates have a diameter of less than or equal to about 100 microns, and wherein such
aggregates return to a nanoparticulate drug dispersion upon reconstitution in an aqueous
liquid medium.

41. (Original) The method of claim 40, further comprising adding a diluent to the nanoparticulate
dispersion prior to spray-drying, wherein following spray-drying essentially every diluent particle
contains at least one embedded drug particle and a surface modifier.

42. (Previously presented) A method of making a dry powder nanoparticulate drug aerosol
formulation comprising:

(a) milling under non-pressurized conditions in a non-aqueous medium having a high boiling
point a dispersion comprising the following:

(i) a poorly soluble crystalline drug, wherein by “poorly soluble” it is meant that the
drug has a solubility in the non-aqueous medium of less than about 10 mg/ml,
and

(ii) a surface modifier, to obtain a nanoparticulate drug composition having an
effective average particle size of less than about 1000 nm, meaning at least 50%
of the drug particles have a particle size of less than about 1000 nm, and

- (b) evaporating the non-aqueous medium to obtain a dry powder of spherically shaped aggregates of drug and surface modifier particles, wherein the aggregates have a diameter of less than or equal to about 100 microns, and wherein such aggregates return to nanoparticulate drug particle dispersions upon reconstitution in an aqueous liquid medium.

43. (Previously presented) A method of making an aerosol composition comprising:

- (a) milling under pressurized conditions in a non-aqueous medium a dispersion comprising the following:
 - (i) a poorly soluble crystalline drug, wherein by “poorly soluble” it is meant that the drug has a solubility in the non-aqueous dispersion medium of less than about 10 mg/ml, and
 - (ii) a surface modifier, to obtain a drug having an effective average particle size of less than about 1000 nm, meaning at least 50% of the drug particles have a particle size of less than about 1000 nm;
- (b) evaporating the non-aqueous medium to obtain a dry powder of spherically shaped aggregates of drug and surface modifier particles, wherein the aggregates have a diameter of less than or equal to about 100 microns, and wherein such aggregates return to nanoparticulate drug particle dispersions upon reconstitution in an aqueous liquid medium; and
- (c) formulating the dry powder spherically shaped aggregates into an aerosol composition.

44. (Previously presented) A method of making a dry powder nanoparticulate drug composition comprising:

- (a) forming an aqueous nanoparticulate dispersion of a poorly soluble drug, wherein:

- (i) the dispersion comprises poorly soluble crystalline drug particles, wherein by “poorly soluble” it is meant that the drug has a solubility in the liquid dispersion medium of less than about 10 mg/ml, and wherein the drug particles have an effective average particle size of less than about 1000 nm, meaning at least 50% of the drug particles have a particle size of less than about 1000 nm, and
 - (ii) a surface modifier adsorbed on the surface thereof; and
- (b) freeze-drying the nanoparticulate dispersion to form a dry powder of spherically shaped aggregates of the nanoparticulate drug and surface modifier particles, wherein the aggregates have a diameter of less than or equal to about 100 microns, and wherein such aggregates return to nanoparticulate drug particle dispersions upon reconstitution in an aqueous liquid medium.

45. (Original) The method of claim 44, further comprising adding a diluent to the nanoparticulate dispersion prior to freeze-drying, wherein following freeze-drying essentially every diluent particle contains at least one embedded drug particle and a surface modifier.

46. (Cancelled).

47. (Original) A method of administering the aerosol of claim 11 to a patient, wherein the aerosol comprises drug at a concentration of 10 mg/g or greater, and wherein the patient delivery time for the aerosol administration is about 15 seconds or less.

48. (Original) A method of administering the aerosol of claim 23 to a patient, wherein the aerosol comprises drug at a concentration of 10 mg/g or greater, and wherein the patient delivery time for the aerosol administration is about 15 seconds or less.

49. (Original) A method of administering the aerosol of claim 35 to a patient, wherein the aerosol comprises drug at a concentration of 10 mg/g or greater, and wherein the patient delivery time for the aerosol administration is about 15 seconds or less.

50. (Cancelled).

51. (Previously Presented) The aerosol composition of claim 11, wherein the nanoparticulate drug particles have an effective average particle size of less than about 300 nm.

52. (Previously Presented) The aerosol composition of claim 11, wherein the nanoparticulate drug particles have an effective average particle size of less than about 250 nm.

53. (Previously Presented) The aerosol composition of claim 11, wherein the nanoparticulate drug particles have an effective average particle size of less than about 100 nm.

54. (Previously Presented) The aerosol composition of claim 11, wherein the nanoparticulate drug particles have an effective average particle size of less than about 50 nm.

55. (Previously Presented) The aerosol composition of claim 23, wherein the nanoparticulate drug particles have an effective average particle size of less than about 300 nm.

56. (Previously Presented) The aerosol composition of claim 23, wherein the nanoparticulate drug particles have an effective average particle size of less than about 250 nm.

57. (Previously Presented) The aerosol composition of claim 23, wherein the nanoparticulate drug particles have an effective average particle size of less than about 100 nm.

58. (Previously Presented) The aerosol composition of claim 23, wherein the nanoparticulate drug particles have an effective average particle size of less than about 50 nm.

59. (Previously Presented) The aerosol composition of claim 11, wherein at least 70% of the drug particles have a particle size of less than about 1000 nm.

60. (Previously Presented) The aerosol composition of claim 11, wherein at least 90% of the drug particles have a particle size of less than about 1000 nm.

61. (Previously Presented) The aerosol composition of claim 23, wherein at least 70% of the drug particles have a particle size of less than about 1000 nm.

62. (Previously Presented) The aerosol composition of claim 23, wherein at least 90% of the drug particles have a particle size of less than about 1000 nm.

63. (Previously Presented) The aerosol composition of claim 35, wherein at least 70% of the drug particles have a particle size of less than about 1000 nm.

64. (Previously Presented) The aerosol composition of claim 35, wherein at least 90% of the drug particles have a particle size of less than about 1000 nm.

65. (Previously Presented) The aerosol composition of claim 42, wherein at least 70% of the drug particles have a particle size of less than about 1000 nm.

66. (Previously Presented) The aerosol composition of claim 42, wherein at least 90% of the drug particles have a particle size of less than about 1000 nm.

67. (Previously Presented) The aerosol composition of claim 43, wherein at least 70% of the drug particles have a particle size of less than about 1000 nm.

68. (Previously Presented) The aerosol composition of claim 43, wherein at least 90% of the drug particles have a particle size of less than about 1000 nm.

69. (Previously Presented) The aerosol composition of claim 44, wherein at least 70% of the drug particles have a particle size of less than about 1000 nm.

70. (Previously Presented) The aerosol composition of claim 44, wherein at least 90% of the drug particles have a particle size of less than about 1000 nm.

71. (Previously Presented) The aerosol composition of claim 19, wherein the drug is selected from the group consisting of proteins, peptides, elastase inhibitors, analgesics, cystic-fibrosis therapies, asthma therapies, emphysema therapies, respiratory distress syndrome therapies, chronic bronchitis therapies, chronic obstructive pulmonary disease therapies, organ-transplant rejection therapies, therapies for tuberculosis and other infections of the lung, fungal infection therapies, and respiratory illness therapies associated with acquired immune deficiency syndrome, an oncology drug, an anti-emetic, and a cardiovascular agent.

72. (Previously Presented) The aerosol composition of claim 19, wherein the nanoparticulate drug particles have an effective average particle size selected from the group consisting of less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 100 nm, and less than about 50 nm.

73. (Previously Presented) The aerosol composition of claim 20, wherein the drug is selected from the group consisting of proteins, peptides, elastase inhibitors, analgesics, cystic-fibrosis therapies, asthma therapies, emphysema therapies, respiratory distress syndrome therapies, chronic bronchitis therapies, chronic obstructive pulmonary disease therapies, organ-transplant rejection therapies, therapies for tuberculosis and other infections of the lung, fungal infection therapies, and respiratory illness therapies associated with acquired immune deficiency syndrome, an oncology drug, an anti-emetic, and a cardiovascular agent.

74. (Previously Presented) The aerosol composition of claim 20, wherein the nanoparticulate drug particles have an effective average particle size selected from the group consisting of less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 100 nm, and less than about 50 nm.

75. (Previously Presented) The aerosol composition of claim 22, wherein the drug is selected from the group consisting of proteins, peptides, elastase inhibitors, analgesics, cystic-fibrosis therapies, asthma therapies, emphysema therapies, respiratory distress syndrome therapies, chronic bronchitis therapies, chronic obstructive pulmonary disease therapies, organ-transplant rejection therapies, therapies for tuberculosis and other infections of the lung, fungal infection therapies, and respiratory illness therapies associated with acquired immune deficiency syndrome, an oncology drug, an anti-emetic, and a cardiovascular agent.

76. (Previously Presented) The aerosol composition of claim 22, wherein the nanoparticulate drug particles have an effective average particle size selected from the group consisting of less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 100 nm, and less than about 50 nm.

77. (Previously Presented) The aerosol composition of claim 30, wherein the drug is selected from the group consisting of proteins, peptides, elastase inhibitors, analgesics, cystic-fibrosis therapies, asthma therapies, emphysema therapies, respiratory distress syndrome therapies, chronic bronchitis therapies, chronic obstructive pulmonary disease therapies, organ-transplant rejection therapies, therapies for tuberculosis and other infections of the lung, fungal infection therapies, and respiratory illness therapies associated with acquired immune deficiency syndrome, an oncology drug, an anti-emetic, and a cardiovascular agent.

78. (Previously Presented) The aerosol composition of claim 30, wherein the nanoparticulate drug particles have an effective average particle size selected from the group consisting of less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 100 nm, and less than about 50 nm.

79. (Previously Presented) The aerosol composition of claim 31, wherein the drug is selected from the group consisting of proteins, peptides, elastase inhibitors, analgesics, cystic-fibrosis therapies, asthma therapies, emphysema therapies, respiratory distress syndrome therapies, chronic bronchitis therapies, chronic obstructive pulmonary disease therapies, organ-transplant rejection therapies, therapies for tuberculosis and other infections of the lung, fungal infection therapies, and respiratory illness therapies associated with acquired immune deficiency syndrome, an oncology drug, an anti-emetic, and a cardiovascular agent.

80. (Previously Presented) The aerosol composition of claim 31, wherein the nanoparticulate drug particles have an effective average particle size selected from the group consisting of less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 100 nm, and less than about 50 nm.

81. (Previously Presented) The aerosol composition of claim 33, wherein the drug is selected from the group consisting of proteins, peptides, elastase inhibitors, analgesics, cystic-fibrosis therapies, asthma therapies, emphysema therapies, respiratory distress syndrome therapies, chronic bronchitis therapies, chronic obstructive pulmonary disease therapies, organ-transplant rejection therapies, therapies for tuberculosis and other infections of the lung, fungal infection therapies, and respiratory illness therapies associated with acquired immune deficiency syndrome, an oncology drug, an anti-emetic, and a cardiovascular agent.

82. (Previously Presented) The aerosol composition of claim 33, wherein the nanoparticulate drug particles have an effective average particle size selected from the group consisting of less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 100 nm, and less than about 50 nm.

83. (Previously Presented) The aerosol composition of claim 35, wherein the drug is selected from the group consisting of proteins, peptides, elastase inhibitors, analgesics, cystic-fibrosis therapies, asthma therapies, emphysema therapies, respiratory distress syndrome therapies, chronic bronchitis therapies, chronic obstructive pulmonary disease therapies, organ-transplant rejection therapies, therapies for tuberculosis and other infections of the lung, fungal infection therapies, and respiratory illness therapies associated with acquired immune deficiency syndrome, an oncology drug, an anti-emetic, and a cardiovascular agent.

84. (Previously Presented) The aerosol composition of claim 35, wherein the nanoparticulate drug particles have an effective average particle size selected from the group consisting of less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 100 nm, and less than about 50 nm.

85. (Previously Presented) The aerosol composition of claim 35, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of about 2 to about 10 microns.

86. (Previously Presented) The aerosol composition of claim 85, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of about 2 to about 6 microns.

87. (Previously Presented) The aerosol composition of claim 35, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of less than about 2 microns.

88. (Previously Presented) The aerosol composition of claim 35, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of about 5 to about 100 μm .

89. (Previously Presented) The aerosol composition of claim 88, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of about 30 to about 60 μm .

90. (Previously Presented) The method of claim 40, wherein the drug is selected from the group consisting of proteins, peptides, elastase inhibitors, analgesics, cystic-fibrosis therapies, asthma therapies, emphysema therapies, respiratory distress syndrome therapies, chronic bronchitis therapies, chronic obstructive pulmonary disease therapies, organ-transplant rejection therapies, therapies for tuberculosis and other infections of the lung, fungal infection therapies, and respiratory illness therapies associated with acquired immune deficiency syndrome, an oncology drug, an anti-emetic, and a cardiovascular agent.

91. (Previously Presented) The method of claim 40, wherein the nanoparticulate drug particles have an effective average particle size selected from the group consisting of less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 100 nm, and less than about 50 nm.

92. (Previously Presented) The method of claim 40, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of about 2 to about 10 microns.

93. (Previously Presented) The method of claim 92, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of about 2 to about 6 microns.

94. (Previously Presented) The method of claim 40, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of less than about 2 microns.

95. (Previously Presented) The method of claim 40, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of about 5 to about 100 μm .

96. (Previously Presented) The method of claim 95, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of about 30 to about 60 μm .

97. (Previously Presented) The method of claim 42, wherein the drug is selected from the group consisting of proteins, peptides, elastase inhibitors, analgesics, cystic-fibrosis therapies, asthma therapies, emphysema therapies, respiratory distress syndrome therapies, chronic bronchitis therapies, chronic obstructive pulmonary disease therapies, organ-transplant rejection therapies, therapies for tuberculosis and other infections of the lung, fungal infection therapies, and respiratory illness therapies associated with acquired immune deficiency syndrome, an oncology drug, an anti-emetic, and a cardiovascular agent.

98. (Previously Presented) The method of claim 42, wherein the nanoparticulate drug particles have an effective average particle size selected from the group consisting of less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 100 nm, and less than about 50 nm.

99. (Previously Presented) The method of claim 42, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of about 2 to about 10 microns.

100. (Previously Presented) The method of claim 99, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of about 2 to about 6 microns.

101. (Previously Presented) The method of claim 42, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of less than about 2 microns.

102. (Previously Presented) The method of claim 42, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of about 5 to about 100 μm .

103. (Previously Presented) The method of claim 102, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of about 30 to about 60 μm .

104. (Previously Presented) The method of claim 43, wherein the drug is selected from the group consisting of proteins, peptides, elastase inhibitors, analgesics, cystic-fibrosis therapies, asthma therapies, emphysema therapies, respiratory distress syndrome therapies, chronic bronchitis therapies, chronic obstructive pulmonary disease therapies, organ-transplant rejection therapies, therapies for tuberculosis and other infections of the lung, fungal infection therapies, and respiratory illness therapies associated with acquired immune deficiency syndrome, an oncology drug, an anti-emetic, and a cardiovascular agent.

105. (Previously Presented) The method of claim 43, wherein the nanoparticulate drug particles have an effective average particle size selected from the group consisting of less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 100 nm, and less than about 50 nm.

106. (Previously Presented) The method of claim 43, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of about 2 to about 10 microns.

107. (Previously Presented) The method of claim 106, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of about 2 to about 6 microns.

108. (Previously Presented) The method of claim 43, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of less than about 2 microns.

109. (Previously Presented) The method of claim 43, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of about 5 to about 100 μm .

110. (Previously Presented) The method of claim 109, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of about 30 to about 60 μm .

111. (Previously Presented) The method of claim 44, wherein the drug is selected from the group consisting of proteins, peptides, elastase inhibitors, analgesics, cystic-fibrosis therapies, asthma therapies, emphysema therapies, respiratory distress syndrome therapies, chronic bronchitis therapies, chronic obstructive pulmonary disease therapies, organ-transplant rejection therapies, therapies for tuberculosis and other infections of the lung, fungal infection therapies, and respiratory illness therapies associated with acquired immune deficiency syndrome, an oncology drug, an anti-emetic, and a cardiovascular agent.

112. (Previously Presented) The method of claim 44, wherein the nanoparticulate drug particles have an effective average particle size selected from the group consisting of less than about 400 nm, less than about 300 nm, less than about 250 nm, less than about 100 nm, and less than about 50 nm.

113. (Previously Presented) The method of claim 44, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of about 2 to about 10 microns.

114. (Previously Presented) The method of claim 113, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of about 2 to about 6 microns.

115. (Previously Presented) The method of claim 44, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of less than about 2 microns.

116. (Previously Presented) The method of claim 44, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of about 5 to about 100 μm .

117. (Previously Presented) The method of claim 116, wherein the aggregates of the nanoparticulate drug particles have a mass median aerodynamic diameter of about 30 to about 60 μm .

118. (Previously Presented) The aerosol composition of any one of claims 14, 25, 71, 73, 75, 77, 79, 81, or 83 wherein the drug is selected from the group consisting of a bronchodilator, a corticosteroid, and an anti-fungal.

119. (Previously Presented) The method of any one of claims 90, 97, 104, and 111, wherein the drug is selected from the group consisting of a bronchodilator, a corticosteroid, and an anti-fungal.

120. (Previously Presented) The aerosol composition of claim 14, wherein the drug is selected from the group consisting of beclomethasone dipropionate, naproxen, triamcinolone acetonide, budesonide, and an anti-emetic.

121. (Previously Presented) The aerosol composition of claim 25, wherein the drug is selected from the group consisting of beclomethasone dipropionate, naproxen, triamcinolone acetonide, budesonide, and an anti-emetic.

EVIDENCE APPENDIX

Chapter 6

AEROSOL FORMULATION, GENERATION, AND DELIVERY USING NONMETERED SYSTEMS

Peter R. Byron

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I. METERED VS. NONMETERED SYSTEMS

Devices which can be used to generate inhalation aerosols fall into two main categories: those which purport to meter the drug dose provided to the patient and those which do not. Because the formulation of metered dose inhalers or MDIs presents the pharmaceutical scientist with some unique challenges, these devices are described separately in another chapter. The present chapter attempts to come to grips with the principles of operation of continuous aerosol generators and their use in the treatment of human disease. These devices, of which nebulizers are the most common example, are used widely by pharmacologists involved with drug testing in animals and are increasingly seen as a useful adjunct to MDI therapy in humans. Despite the implication in the chapter title that nebulizers provide an unknown dose, they can be used conveniently by researchers who seek to establish the dosimetry of compounds delivered to the lungs. This step is a necessity in the development phase for a new drug entity and should be performed prior to the formulation and design of metered dose units. While the majority of this chapter is concerned with the use of continuous aerosol generators to administer drugs in a therapeutic setting, two sections (I.L.C. and V.C.) address the subject of assigning a value for "dose to the lung" applicable to a drug development protocol.

Some of the advantages and disadvantages of nonmetered systems such as portable nebulizers are listed in Table 1. In view of the fact that aerosols are presently inhaled mostly by breathing-impaired patients, the predominant factor dictating the use of a nebulizer over an MDI is patient preference. Nebulizers offer the possibility of tidal inhalation and exhalation (as opposed to deep inhalation and breath-hold, phased with actuation for optimal MDI usage). However, in view of the fact that some new compounds may be administered to lung-normal patients (for systemic delivery perhaps), this factor is drug and disease specific. From a technological point of view, the possibility of administering much larger doses by employing continuous aerosol inhalation is the nebulizer's most appealing advantage. Disadvantages, on the other hand, are many; the level of patient education is critical if hygiene is to be maintained and, because of the complexity associated with these devices, much of the control remains in the hands of the patient. The wide variety of available devices and their different specifications makes misuse a likely occurrence.

II. ESTIMATING DOSIMETRY

Only a basic understanding of the factors affecting aerosol deposition in the lung is necessary in order to realize that neither continuous generators nor so-called "metered dose inhalers" really control the drug dose reaching the airways of the lung. It is true that the MDI limits the amount a patient can inhale from a single actuation of the inhaler, but in neither this case, nor that of the nebulizer, will all of the aerosolized material reach and deposit in the lung. While it is not the intention to review deposition in detail (see Chapter 1), the importance of defining dosimetry in terms of drug mass requires that a simple mass-based deposition model be presented here as a precursor to later arguments to be used in this and the next chapter.

A. STABLE AEROSOLS INHALED BY NORMAL HUMANS

When discussing the different categories of generation and delivery device, it is helpful to keep a simple model of aerosol deposition in mind. Aerosol size distribution is the single most important variable in defining the size of droplet or particle deposition in the patient; in short, it will determine whether drug targeting succeeds or fails. From a pharmaceutical point of view, the most important parameter is usually the mass median aerodynamic diameter (MMAD) of an aerosol. This is the aerodynamic diameter above and below which 50% of the mass resides. Aerodynamic diameter is the diameter of a unit density sphere which behaves in air in the same way as the droplet or particle in question. In the frequent event that MMAD is determined by

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TABLE I
Advantages and Disadvantages of Nonmetered Aerosol Delivery Devices

| Advantages | Disadvantages |
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| 1. Large expiratory dose | 1. Complexity |
| 2. Patient preference | 2. Expense and portability |
| 3. Wear is usually the solvent | 3. Hygiene considerations |
| 4. Patient coordination is not usually a problem | 4. Devices and techniques are nonstandardized |

- * In the home, in public places, smaller devices like MDIs are usually preferred.
* Propulsion and expiratory velocity is not an issue.

fractionating the aerosol according to droplet size and then analyzing drug content in each of the fractions, then the term reflects the size above and below which 50% of the drug mass resides.¹ Because the concentration of drug may be different in small and large droplets, this is not necessarily the same as the MMAD of the aerosol itself. Nevertheless, it is this drug MMAD which matters here because this will define how the drug mass will deposit in the lung.² It is important when reading the literature to realize that the mass median diameter is often about three to four times larger than either the count or number mean or median diameters.³ The number median, for example, is the diameter above and below which 50% of the number of droplets resides. The latter value is smaller than the MMAD because a single 10 μm sphere has 1000 times the mass of one with a diameter of 1 μm . Articles in the medical literature and device manufacturers frequently state count mean diameters to describe the output of different devices which make the aerosols sound much better for inhalation than they really are.⁴ Diameters other than aerodynamic are sometimes also quoted. The difference between actual and aerodynamic diameters, d_p , which are related through the density and shape of the particle or droplet, is given for spheres with diameters greater than $\approx 1 \mu\text{m}$ by

$$d_p = (\text{actual diameter}) \cdot (\text{density})^{1/2} \quad (1)$$

where density is expressed in gcm^{-3} . This relationship is less important for aqueous aerosols than it is for aerosolized pharmaceutical powders where densities are often in the range of 1 to 2 gcm^{-3} .⁵ Readers should review a simple text on particle size analysis⁶ in order to clarify their understanding of these points. Provided an aerosol is log-normally distributed and the geometric standard deviation is known, it is possible to relate the different mean and median diameters analytically. Even so, because therapeutic aerosols often deviate from log-normality and sometimes display polymodal distributions, it is better to measure the MMAD itself rather than try to calculate it from some other value.⁶

Different aerosol size distributions may be expected to deposit preferentially in different regions of the respiratory tract. Aerosols inhaled through the nose have different deposition patterns to those inhaled orally.⁷ In Figure 1, oral inhalation by normal subjects is assumed because this is the more frequent mode of inhalation. Fractional deposition (in terms of particle or droplet mass) is shown for monodisperse, stable aerosols. The data in support of Figure 1 are derived from human exposure to radiolabeled dust aerosols with subjects inhaling and exhaling tidally.¹² If the horizontal axis on Figure 1 were labeled "mass median aerodynamic diameter" so that polydisperse aerosols were considered,⁸ the curves indicating deposition in the lung may be reduced in height, indicating less efficient deposition, but the maxima would remain at the same diameters.¹⁰ In normal subjects, deposition in the pulmonary or alveolar compartment is optimal for aerosols with aerodynamic diameters around 2 to 3 μm provided inhalation is slow (20 to 30 l/min).¹¹ Maximum deposition in the tracheobronchial regions occurs with slightly

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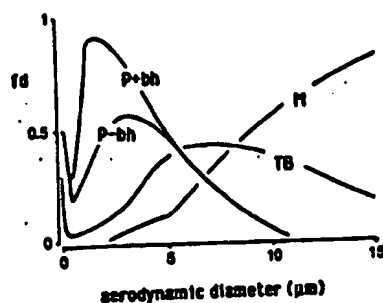


FIGURE 1. Standard fractional mass deposition, f_d , vs. aerodynamic diameter (μm) in oropharynx or mouth (M), tracheobronchial region (TB), and pulmonary region with ($P+th$) and without breath-hold ($P-th$). Deposition is for stable monodisperse aerosols inhaled by normal human breathing steadily with inspiratory flow rates ~ 42 L/min. Polydisperse aerosols produce similar curves with lower maxima when mass median aerodynamic diameter is plotted on the horizontal axis. (Reproduced with permission of the American Pharmaceutical Association.)

larger particles, although mucociliary clearance can remove material quite quickly from this region (Chapter 6).

B. BREATHING PATTERN AND DISEASE

The curve maxima in Figure 1 shift on both axes if the breathing pattern is changed. With faster inhalation, smaller aerosols usually deposit higher in the respiratory tract (tracheobronchial deposition is enhanced) than would be the case if inhalation were slow. In part this is due to increased turbulence. The tracheobronchial or conducting airways extend down to the terminal bronchioles.⁹ Constriction of the tracheobronchial airways is responsible for most of the breathing difficulty in reversible asthma. Unlike the pulmonary region, these airways have smooth muscle in their walls. It is obvious that aerosol deposition in an asthmatic individual will be different from that in a normal subject. Partly due to turbulence in constricted airways but also because the aerosol cannot penetrate well into poorly ventilated areas, deposition tends to occur more centrally in patients than it does in normal subjects.¹² Also, intersubject variations in deposition are much larger in the diseased population. This variability in aerosol deposition, which the pathophysiology of asthma creates,¹² obliterates many of the finer differences resulting from aerosol size changes and breathing pattern. Even so, the trends shown in Figure 1 remain broadly true. Aerosol particles with larger aerodynamic diameters have an increased tendency to collide with surfaces in their paths and separate by impaction in the upper airways. Smaller particles (which form more stable aerosols) tend to separate largely by sedimentation in the small, peripheral airways provided the airflow takes them there. The upper curve in Figure 1 shows an estimate of the increased deposition in the lung periphery resulting from breath-holding after inhalation.¹¹ If small particles ($\sim 2 \mu\text{m}$; Figure 1) do not reach the periphery, where the sedimentation distances become small enough for them to deposit during a breath-holding pause, then they are more likely to be exhaled.^{14,15} In the bronchoconstricted asthmatic, when airway narrowing is substantial, some small enhancement in deposition due to settling may be expected in the upper airways as a result of breath-holding.

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C. PRINCIPLES OF DOSE ESTIMATION

Presumably, the drug formulator and product designer wish to deliver a known dose more or less reliably to the lungs of a patient. If we assume at this stage that it is possible to generate several tidal volumes of homogeneously distributed aerosol and make this available at the subject's mouth, in principle it is then possible for the formulator to estimate the dose administered to the lungs. Several basics should be standardized and have known values. These are

1. Drug concentration in air
2. Aerosol size distribution (drug MMAD and a measure of polydispersity)
3. The subject's breathing pattern (rate and frequency of inspiration, duration of breath-hold, and inhaled volume)

Then, let us assume that a well-trained, "lung-normal" subject inhales at a known rate, say 20 to 30 l/min, and breath-holds for a chosen interval. Assume further that he or she inhales a tidal volume of aerosol equal to 3 l on 4 separate inhalations. If the aerosol size is known, then it is usually possible to choose an appropriate deposition model and assign a value to mass fractional deposition, f_d (Figure 1). This may be in the whole lung (TB + P; Figure 1) or a portion of it, as described in an earlier chapter, whichever is considered to be most important. The product of the aerosol concentration and inhaled volume then provides an estimate of the drug mass inhaled which, when multiplied by f_d , gives the estimated dose deposited. While some of the deficiencies of this approach are discussed later in Section V.C, it is apparent that the aerosol formulator and device designer must work closely together to produce a combination which delivers drug in aerosolized form with an appropriate concentration and particle size distribution so as to optimize its mass deposition in the respiratory tract. The aerosol concentration and size distribution which matters is the one which the patient sees at his or her oropharynx, and is not necessarily the one which was determined perhaps 1 m of tubing before reaching the patient. Although it is difficult to achieve, the ideal aerosol should be presented to the patient as a stationary, stable cloud of particles or droplets suspended in air.

III. DYNAMICS OF INHALATION AEROSOLS

An understanding of three important phenomena which affect aerosol size and concentration are required prior to explaining the performance characteristics of different devices. An understanding of particle or droplet impaction, sedimentation, and solvent evaporation or condensation kinetics is essential to the design of successful administration systems. These three phenomena (impaction, sedimentation, and the size changes induced by evaporation or condensation) stand out from others in aerosol physics (thermophoresis, photophoresis, and particle diffusion, for example) as being the primary mechanisms by which aerosolized medicaments separate and/or change their particle size distributions most rapidly within delivery systems. Each topic is described briefly in turn.

A. DROPLET OR PARTICLE IMPACTION

Aerosol segregation on the basis of the inertial properties of the dispersed phase is important not only to explain deposition in the upper airways (Chapter 1), but also to understand some of the principles by which inhalation devices function and, in some cases, fail to function. It is necessary to gain a simple understanding of the process (which is also used as the principle of operation for cascade impactors) sufficient to be able to say when droplet impaction is or is not likely to occur. Figure 2 is a schematic showing aerosol passage within a circular tube of internal diameter, W . The large droplet is shown leaving the airflow and impacting while the smaller one deviates with the airflow. In order to simplify the theory, the distance from the end of the tube

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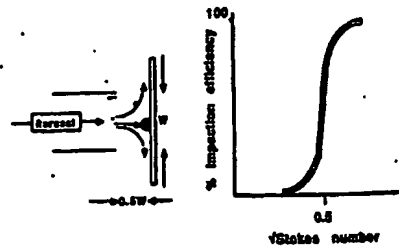


FIGURE 2. Diagrammatic representation of particle impaction on an aerosol stream impinging on a horizontal plate in its path. Impaction efficiency is close approximately 50% when $\sqrt{\text{Stokes' number}} = 0.5$.

to the impaction surface is held equal to $1/2$ the width of the tube, W , and is thus equal to the maximum distance a particle or droplet traveling down its center must deviate from the airflow in order to collide with the surface in its path. Figure 2 also shows a typical plot of impaction efficiency as a function of the dimensionless Stokes' number, S_{dt} , which, for unit density spheres $> 1 \mu\text{m}$ in diameter, in air at 1 atm and 20°C , can be written in the centimeter-gram-second system as:

$$S_{dt} = V^2 d^2 / (0.00165 W) \quad (2)$$

where V is the linear velocity of the airflow and d is droplet diameter. Because $\sqrt{S_{dt}}$ is often ≈ 0.5 ($S_{dt} = 0.25$) for a 50% probability of impaction occurring (Figure 2), it is a simple matter to calculate the approximate linear velocity at which a droplet of known diameter will impact in an arrangement like that shown in Figure 2. If, for example, W were 1 cm and $d = 5 \mu\text{m}$ (or 5×10^{-4} cm) then the critical velocity for a 50% chance of impaction would be

$$V_{50} = 0.25 \times 0.00165 \times 1 = 1650 \text{ cm/s} \quad (3)$$

$$(5 \times 10^{-4})^2$$

or 16.5 m/s. In order to increase impaction of smaller droplets in this set up, greater velocities are necessary. Alternatively, the value of $0.5 \times W$ (the distance the droplet needs to deviate from the airflow) can be made smaller. Calculations of this type are relevant to baffle design in jet nebulizers and particle deposition from MDIs. They can also be used to explain why impaction is only a major mechanism of deposition in the upper airways of the lung where the linear airflow velocities remain high.

B. SEDIMENTATION

Table 2 shows sedimentation velocities of unit density spheres in still air at 20°C . The values only assume meaning when used in conjunction with aerosol residence times in containers of known dimensions. Consider, for example, an aerosol flowing horizontally at 5 l/min along a 30 cm tube with an internal diameter equal to 2 cm. The linear airflow velocity is given by (volume flow rate)/(area) or $(5000 \text{ cm}^3 \text{ min}^{-1}) / 1 \text{ cm}^2 = 1592 \text{ cm min}^{-1}$. The residence time in 30 cm of tubing ($30 \text{ cm} / 1592 \text{ cm min}^{-1} = 0.0188 \text{ min}$) is thus 1.13 s. Because a $10\text{-}\mu\text{m}$ sphere can settle 0.35 cm in this residence time, about 18% of $10 \mu\text{m}$ spheres will be deposited in the tube during the passage of the aerosol. This percentage is a function of droplet size and can be

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TABLE 2
Sedimentation Velocities of Unit Density Spheres in
Dry Air at 20°C and 1 atm Pressure

| Aerodynamic diameter (μm) | Sedimentation velocity (cm/s) |
|--|-------------------------------------|
| 2.0 | 0.013 |
| 4.0 | 0.050 |
| 6.0 | 0.111 |
| 8.0 | 0.197 |
| 10.0 | 0.306 |
| 20.0 | 1.212 |

estimated quite simply from the ratio of distance settled to the residence time to the internal diameter of the tubing. Although these arguments neglect to account for turbulent mixing, they can be extended to their logical conclusions which are frequently found in practice to be correct. There are three main results of passing polydispersed aerosols in our size range of interest (Figure 1) along tubes. The segregation induced by the sedimentation process ensures that the aerosol flowing from the tubing outlet has (1) decreased concentration, (2) a smaller median diameter, and (3) decreased "polydispersity". The relative magnitude of all these segregation effects becomes smaller as the aerosol droplet or particle size is reduced as the inlet to the tube because the aerosol becomes "more stable" and is less likely to sediment quickly. The "polydispersity" of the aerosol distribution refers to the magnitude of the spread of the particle size distribution. It is frequently characterized numerically as the geometric standard deviation (GSD) of log-normally distributed aerosols.⁹ Some of these effects are shown in our work designed to determine drug absorption kinetics after characterized aerosol administration to dogs.^{14,15} As they were generated, the aerosols of three median sizes were all significantly polydispersed (GSD = 2). After passage along the tubes required for administration by positive pressure ventilation, however, segregation caused the larger aerosols (3.5 and 4 μm MMAD) to tend toward monodispersity (GSD = 1.3). This effect was much less for the smallest aerosol (1.0 μm MMAD) which was administered after passage through the same apparatus, with a GSD equal to 1.6.¹⁵

C. EVAPORATION AND CONDENSATION

Therapeutic aerosols are unstable in two major respects. First, they contain droplets or particles which are too large to stay in suspension for long. Second, they contain volatile or hygroscopic materials which cause aerosol size changes to occur as a function of time, temperature, aerosol dilution, and droplet or particle content. The thermodynamics and kinetics of this latter subject are too complex to consider in detail here, even when single particles or droplets are considered. In simple terms, and as far as therapeutic aerosols are concerned, two questions are of importance:

1. How fast can evaporation or condensation alter the particle size distribution of an aerosol?
2. How much can they change the size distribution (if they occur fast enough)?

The enormous surface:volume ratio presented by typical medicinal aerosols requires that we think of water as a volatile material and thus, a reasonable answer to the first question is "fast enough to worry about". The calculated lifetime of a 10- μm water droplet at 25°C and 50% relative humidity is only $\sim 0.6 \text{ s}$ ¹⁶ and a breath of aerosol usually takes several seconds to inhale.¹ Furthermore, and with obvious relevance to MDIs, some of the solvents and propellants used

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In therapeutic aerosols are more volatile than water. Thus, as a rule of thumb, we can often assume that aerosols adjust their size distributions extremely quickly. To answer the second question, droplets will grow or shrink until their solvent vapor pressures are in equilibrium with the environment. Different droplet "environments" may display a wide range of temperatures but rarely contain fluorocarbon or ethanol vapors in significant concentrations. While ingredients like these *should* be lost rapidly and completely, we will see the complexity of the subject in the following chapter, when we observe that inadequate heat transfer to supercooled propellant droplets ultimately slows and governs the rate at which the size of MDI generated aerosols can shrink to the respirable range.

Because atmospheric air, compressed air, and the air in the lung contain water vapor, the behavior of aqueous aerosols can vary as a function of the environmental humidity. Pure water aerosols will obviously tend to evaporate at all relative humidities less than 100%. At constant temperature, aqueous aerosols containing dissolved salts have a particular relative humidity with which they exist in equilibrium. At this relative humidity, which is roughly predictable from a consideration of Raoult's law and a psychrometric chart,¹² they show no tendency to grow or shrink. However, systems which are isotonic with blood are very dilute and tend to evaporate as they pass in dilution air from a nebulizer to the patient. The opposite occurs with some hypertonic solution and dry powder aerosols.¹ These often display hygroscopic growth in the humid environment of the lung.¹³ The relative humidity in the lung is in excess of 99% at 37°C.¹ This relative humidity would be that measured over a solution of isotonic saline at the same temperature. Water-soluble drugs administered as hypertonic solutions or solids, therefore, tend to grow rapidly as they pass through the humid environment of the respiratory tract.^{13,14} The size they try to attain during inhalation is defined theoretically by the aqueous droplet size which would contain a drug concentration isotonic with blood. These concentrations are often of the order of 1 to 5% showing that the growth tendency of a typical hygroscopic solid is to take on about 20 to 100 times its own mass of water (employing hypertonic aerosols to enhance deposition in the lung has been advocated recently in the literature¹⁵). Because the diameter of a droplet is proportional to the cube root of its volume (and ignoring density considerations), a 20-fold growth in mass would correspond to an approximate growth ratio of (equilibrium diameter at lung humidity)/(dry particle diameter) = $20^{1/3}$ or 2.7. In practice during inhalation, ratios less than those predicted at equilibrium are operative because the process is dynamic. Nevertheless, this discussion shows the importance of paying careful attention to the various humidities which can be encountered in administration systems as well as the hygroscopic tendencies of some solid materials.

IV. AEROSOL GENERATION

There are two major types of nonmetered inhalation device which are used largely for the treatment and prevention of respiratory disease. These are jet or ultrasonic nebulizers. In practice, neither of these have been well accepted for the delivery of therapeutic agents for systemic purposes. Systemic administration potential clearly exists, however, given the wide acceptance of this route for the administration of drugs of abuse. Inhalation offers rapid absorption opportunities for compounds which require fast onset. Furthermore, the anatomical arrangements of the major vessels are such that they could usefully be employed to target absorbed compounds to the heart.

Mainly over the last decade, the design and construction of devices has been improved in order to enhance the drug doses which can be delivered successfully to the breathing-impaired. The main improvements have been to increase the output of respirable aerosol. This has been achieved in two ways: first, by increasing total output concentrations and secondly, by reducing droplet or particle sizes emitted by nebulizers. Because of recent improvements in aerosol dust-generator design and the usefulness of these latter devices for animal experimentation and

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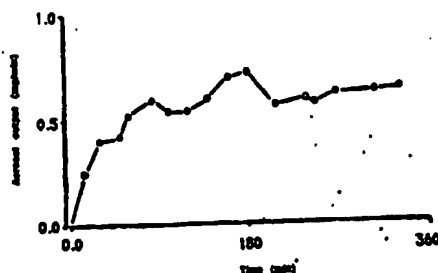


FIGURE 3. Mass of micronized aluminum flowable powder emitted per minute as dry powder aerosol from a Model 3400 fluidized bed aerosol generator (TSI, St. Paul, MN) supplied with dry air at 10 l/min. Aerosol concentrations approach constancy after approximately 3 h when conveyed powder input to the fluid bed is balanced by aerosol output from the generator.

toxicity testing, the following discussion includes three major device categories. These are discussed in turn alongside some of the accessories which are commonly used with them: (1) dry powder generators, (2) air-blast nebulizers, and (3) ultrasonic nebulizers.

A. DRY POWDER GENERATORS

Dust generators have long been in existence, the most famous of which is probably the Wright Dust-Feed Apparatus.²¹ However, these generators have historically been difficult to adapt to pharmaceutical studies. More recently, fluidized bed technology has improved to such an extent that generators are now available commercially which are capable of deaggregating powder charges and providing these as dispersions of powders in air.^{14,22} The dispersed dust can often be shown to possess the same size characteristics as the powder with which the bed was supplied.²³ Thus, it is possible to micronize pharmaceutical powders, pass these into a bed of metal beads which are fluidized by dry air or gas which causes the beads to appear to "boil", and utilize the aerosol formed by the deaggregating action of the bed. Provided the bed is fed with powder continuously, a steady state is reached between powder input and aerosol output which demands that the aerosol output remain at a constant concentration (Figure 3). Steady-state concentrations of respirable aerosol ($<5 \mu\text{m}$ aerodynamic diameter), which can approach 1 mg/l under some circumstances, are several times higher from these devices than those which can be produced by other generators.¹⁴ The high concentrations are important for reasons described below. The devices themselves and their utilization have been described in more detail elsewhere.^{14,23} They are manufactured and marketed by TSI (St. Paul, MN).

Performance of inhalation dosimetry and toxicity studies usually requires dose ranging and is often severely limited by the maximum available aerosol concentration emitted by the chosen generator. This is especially true for pharmaceutical studies where it is important that the stability of the administered compound is maintained during aerosol generation. Condensation generators, for example, require vaporization of the chemical agent prior to its condensation as a high concentration aerosol.²³ Such a procedure is usually unacceptable in pharmaceutical trials.²⁴ The selection of a generator which provides the greatest output concentration is important for two main reasons. First, and most important, respiratory (as opposed to systemic) toxicity is extremely difficult to detect, yet it remains important, even in phase I studies of new

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1.1 This test method specifies a method for the determination of tap density (packed density) of metallic powders and compounds, that is, the density of a powder that has been tapped, to settle contents, in a container under specified conditions.

1.2 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

B212 Test Method for Apparent Density of Free-Flowing Metal Powders

B215 Practice for Sampling Finished Lots of Metal Powders

B243 Terminology of Powder Metallurgy

B329 Test Method for Apparent Density of Powders of Refractory Metals and Compounds by Scott Volumeter

B417 Test Method for Apparent Density of Non-Free-Flowing Metal Powders

B703 Test Method for Apparent Density of Metal Powders Using the Arnold Meter

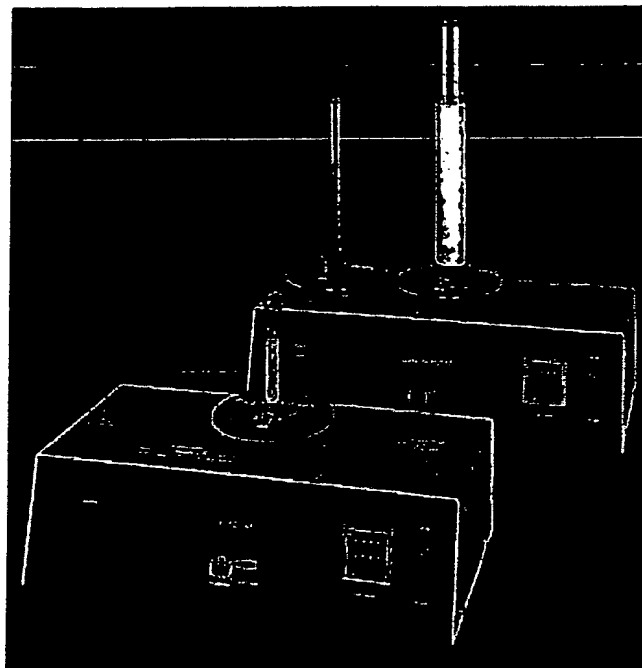
Index Terms

apparent density; bulk density; density; density ratio; metal powders; packed density; powder metallurgy; tap density

Autotap and Dual Autotap

The apparent densities of powdered, granular or flaked materials are highly dependent on the manner in which the particles are packed together. During tapping, smaller particles distribute into the spaces between larger particles. Gradually particles pack more efficiently, the powder volume decreases and the tap density increases, often by 50% to 100%.

For this measurement, Quantachrome has developed two instruments which conform to the British Pharmacopoeia method for Apparent Volume and ASTM standard test methods B527, D4164 and D4781 for Tap Density. These two models, the Autotap and the two sample Dual Autotap, automate the required procedures to provide accurate, reproducible values.



Samples are placed in standard graduated cylinders and mounted on a universal tap platform designed to accommodate cylinders from 10 ml to 1000 ml. Rotation of the tap platform facilitates reading by keeping a flat interface. After noting the initial volume and weight of the material, tapping is started.

If the material characteristic is unknown, tapping may be done continuously, or step-wise by user specified numbers of taps, while noting or graphing the results until the volume becomes constant. In either case the number of taps is displayed. Once the material characteristic is known the proper number of taps, typically thousands, can be preset on subsequent runs thus freeing the operator for other work.

Specifications

| | | | | | |
|------------------------|------------------|-------------|--------|-------|--------|
| Graduated Cylinders(s) | 250 ml* | Dimension | Length | Width | Height |
| Cam Shaft Speed | 260 rpm | inches | 11 | 21 | 6 |
| Tap Stroke Travel | 0.125 inch, 3.18 | centimeters | 28 | 54 | 15 |
| Electrical | 115 V, 60 Hz | Weight | SINGLE | | DUAL |
| Tap Counter | 4 digit (0-9999) | pounds | 25.1 | | 27.9 |
| | | kilograms | 11.4 | | 12.7 |

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Optimal Tapped Density Tester



Available in USP or ASTM versions, our dual platform Optimal Tap Density Testers offer a standardized and repeatable method for measuring tapped or packed volumes of powders and granulated or flaked materials. Our microprocessor controlled USP model features a rotating platform for simultaneous tapping and rotation of the cylinder to ensure a level reading. The USP version provides a 5/8-inch vertical drop per tap at 300 taps per minute and includes one 100 ml funnel top graduated cylinder per platform. Our ASTM version (which complies with the American Society for Testing and Materials Standards) features a non-rotating platform, a 1/8-inch vertical drop per tap at 250 taps per minute, and one 250 ml graduated funnel top cylinder per platform. ASTM method D4164-82 is for testing catalyst particles and requires a 250 ml cylinder and platform; B527-93 involves metallic powders and compounds and requires a 100 ml cylinder and platform.

All of our Tap Density Testers have digital LED displays and user selectable counter or timer operation. The embedded sealed membrane keypad is impervious to dust and particles and can be used to calculate density measurements. The units can be programmed to any number of taps or time period required. An optional thermal printer records program test conditions, time, date, and density measurements.

Tap Density Tester, USP 24 / NF 19

Includes dual rotating platform drive units and two 100 ml graduated funnel top cylinders (class B).

400-001 115V, 60Hz, dual platform, USP

400-002 230V, 50Hz, dual platform, USP

Tap Density Tester, ASTM

Includes dual non-rotating platform drive units and two graduated funnel top cylinders (class B).

400-003 115V, 60Hz, dual platform, ASTM with 250 ml graduated cylinders

400-004 230V, 50Hz, dual platform, ASTM with 250 ml graduated cylinders

400-006 115V, 60Hz, dual platform, ASTM with 100 ml graduated cylinders

400-007 230V, 50Hz, dual platform, ASTM with 100 ml graduated cylinders

IAA24 are likely participants in auxin gene regulation through the TGCTC elements. A single copy of ER8 was a more active AuxRE than other constructs that contained two copies of TGCTC (3, 11) and could represent the perfect palindromic AuxRE, similar to the perfect palindromic GRE (4).

As the COOH-terminal $\beta\alpha$ -motif has no apparent effect on ARF1 binding to DNA, what might be its function? We used the COOH-terminal region of ARF1 as bait in a yeast two-hybrid screen (14) and isolated two identical cDNA clones from an *Arabidopsis* cDNA expression library. The translated open reading frame encoded a protein (ARF1-Binding Protein or ARF1-BP) that contained a region with amino acid sequence similarity to boxes III and IV of ARF1 (Fig. 2, A and C). ARF1-BP showed less similarity to boxes III and IV in Aux/IAA and IAA24 proteins. Thus, boxes III and IV in ARF1 may facilitate interaction of ARF1 with ARF1-BP, and these interactions may contribute to auxin responsiveness.

Genetic approaches to dissect the auxin signal transduction pathway have resulted in the cloning of *AXR1*, *AUX1*, and *hookless1* genes (15). Identification of the relevant transcription factors should facilitate elucidation of the mechanisms involved in auxin-regulated gene expression.

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5. The P3(4x) AuxRE was placed upstream of the minimal promoter in pHis1 and pLacZ vectors (MATCH-MAKER One-Hybrid System; Clontech). These vectors were linearized and sequentially transformed into the yeast strain Y4271 (Clontech). Background LacZ activity in late logarithmic-phase cells for the engineered strain Y4271::P3(4x) was higher than for the pLacZ vector (no AuxREs) but low enough to distinguish from a positive interaction. Low plating density (10^6 cells per 150-mm plate) and 3-aminotriazole (3-AT; 45 mM) were used to suppress growth on histidine-deficient (-His) plates resulting from the low amount of P3(4x)-His3 reporter gene expression. An *Arabidopsis* cDNA expression library cloned into the GAL4 activation domain vector pGAD10 (Clontech) was amplified in *Escherichia coli*. Purified library DNA (500 μ g) was used to transform the Y4271::P3(4x) strain. Of 1.2×10^6 transformants, 500 colonies grew on -His plates containing 3-AT, and these were screened for lacZ activity. LacZ-positive colonies (212) were selected, and library plasmids were isolated. Sizes of the cDNA inserts were determined by polymerase chain reaction (PCR) with primers that flanked the cDNA inserts. Plasmids harboring different-sized inserts were rescued by transformation into *E. coli* and retransformed into the Y4271::P3(4x) strain. Clones that restored lacZ activity were sequenced, and five clones encoding ARF1 were recovered.
6. An ARF3 cDNA (GenBank accession number U89526) was isolated from the MATCH-MAKER cDNA library by using an unannotated *Arabidopsis* sequence (GenBank accession number U78721) related to the NH₂-terminal region of ARF1.
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13. The EPD spacing construct was actTGCTCGAGACac. EP1 to EP9 contained spacers between actTGCTC and GAGACac of one to nine nucleotides: a, aa, cag, caag, ccaag, cccaag, ccccaag, cccccag, and cccccag.
14. The COOH-terminus of ARF1 (amino acids [aa] 533 to 685) served as bait to screen the library used in the one-hybrid screen. The interacting clone encoded the COOH-terminal portion of ARF1-BP (aa 273 to 410). Complementary DNA clones encoding NH₂-terminal regions of ARF1-BP cDNA were isolated by PCR.
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18. Histidine-tagged ARF1 was expressed and purified from *E. coli*, full-length and truncated forms of ARF1 were synthesized in vitro in RiboRabbit reticulocyte lysates (Promega), and gel-shift assays were done as described (16).
19. The IAA24 ORF was isolated from the MATCH-MAKER cDNA library by PCR. IAA24 protein was produced by in vitro translation.
20. Supported by NSF grants IBN 9303956 and MCB 9604208. We thank W. Yu and X. Feng for technical assistance.

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Large Porous Particles for Pulmonary Drug Delivery

David A. Edwards,* Justin Hanes, Giovanni Caponetti, Jeffrey Hrkach, Abdelaziz Ben-Jebria, Mary Lou Eskew, Jeffrey Mintzes, Daniel Deaver, Noah Lotan, Robert Langer*

A new type of inhalation aerosol, characterized by particles of small mass density and large size, permitted the highly efficient delivery of inhaled therapeutics into the systemic circulation. Particles with mass densities less than 0.4 gram per cubic centimeter and mean diameters exceeding 5 micrometers were inspired deep into the lungs and escaped the lungs' natural clearance mechanisms until the inhaled particles delivered their therapeutic payload. Inhalation of large porous insulin particles resulted in elevated systemic levels of insulin and suppressed systemic glucose levels for 96 hours, whereas small nonporous insulin particles had this effect for only 4 hours. High systemic bioavailability of testosterone was also achieved by inhalation delivery of porous particles with a mean diameter (20 micrometers) approximately 10 times that of conventional inhaled therapeutic particles.

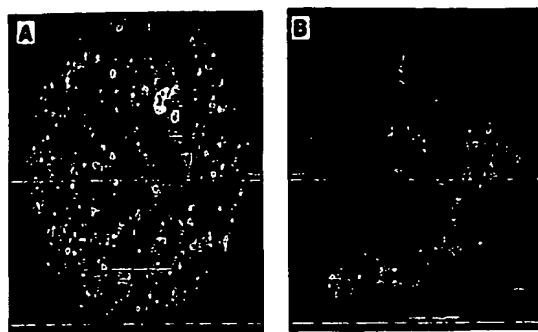
Inhaled aerosols are effective therapeutic carriers for the treatment of respiratory inflammation (1), cystic fibrosis (2), and other lung disorders (3); they also offer potential for non-invasive systemic delivery of peptides and pro-

teins (4). Local and systemic inhalation therapies can often benefit from a controlled release of the therapeutic agent (5), as is achievable with the use of biodegradable polymeric materials (6). Slow release from an inhaled therapeutic particle can prolong the residence of an administered drug in the airways or acini and can diminish the rate of a drug's appearance in the bloodstream (7). Also, patient compliance increases when dosage frequency is reduced (7).

The human lungs, however, have efficient means of removing deposited particles over periods ranging from minutes to hours. In the upper airways, ciliated epithelia contribute to the "mucociliary escalator" (8), by which particles are swept from the airways toward the mouth. In the deep lungs, an army of alveolar macrophages is capable of phagocytosing particles soon after their deposition (9). An effective slow-release inhalation therapy therefore requires a means of avoiding or suspend-

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Fig. 1. Confocal microscopy images of (A) porous PLGA and (B) porous PLAL-Lys particles. Fluorescein isothiocyanate-dextran was encapsulated in the PLGA particle to render the pore spaces of the particle visible in the fluorescent confocal image. The PLAL-Lys particles were fluorescently labeled through the reaction of rhodamine isothiocyanate with lysine amine groups on the surface of the particles. The PLGA and PLAL-Lys particles are highly porous, as evidenced by the appearance of fluorescence throughout the particle structure.



ing the lungs' natural clearance mechanisms until encapsulated drugs have been effectively delivered.

Until now, therapeutic dry powder aerosols have been made with particle mass densities (ρ) of $\sim 1 \pm 0.5 \text{ g/cm}^3$ and mean geometric diameters (d) of $< 5 \mu\text{m}$ to avoid excessive deposition in the dry powder inhaler (DPI) and oropharyngeal cavity (5, 10). Here, we show that very light particles ($\rho < \sim 0.4 \text{ g/cm}^3$) with $d > 5 \mu\text{m}$ can be deposited in the lungs. As a consequence of their large size and low mass density, porous particles can aerosolize from a DPI more efficiently than smaller nonporous particles, resulting in higher respirable fractions of inhaled therapeutics. Also by virtue of their size, large particles can avoid phagocytic clearance from the lungs until the particles have delivered their therapeutic dose; this attribute can be particularly useful for controlled-release inhalation therapies.

To assess the merits of large porous particles for pulmonary drug delivery, we encapsulated model therapeutics inside porous particles (Fig. 1A) composed of 50:50 poly(lactic acid-co-glycolic acid) (PLGA). Double- and single-emulsion solvent evaporation techniques (11) were used to prepare porous and nonporous PLGA particles, respectively. Porous and nonporous particles of similar aerodynamic diameter (12), loaded with ~ 15 weight % model therapeutic (testosterone), were aerosolized into a cascade impactor system (13) from a Spinhaler DPI for 30 s at an airflow rate of 28.3 liter/min. The cascade impactor provides an in vitro system for estimating the respirable fraction of a dry powder; it consists of a closed chamber within which flat plates are arranged perpendicular to the airflow, such that particles deposit stagewise in a manner reflective of their aerodynamic diameters. After deposition on the stages of the impactor, particles were collected (13) and total particle mass was assessed stagewise; the respirable fraction was determined as the percent of total particle mass exiting the DPI, recovered from the terminal, "respirable" stages

of the impactor. Nonporous particles [$d = 3.5 \mu\text{m}$, $\rho = 0.8 \text{ g/cm}^3$ (14)] exhibited a respirable fraction of $20.5 \pm 3.5\%$, whereas $50 \pm 10\%$ of porous particles ($d = 8.5 \mu\text{m}$, $\rho = 0.1 \text{ g/cm}^3$) were respirable, even though the aerodynamic diameters (12) of the two particle types are nearly identical. The large porous particles' high efficiency can be attributed to their smaller surface-to-volume ratio. Large particles aggregate less than small particles, all other considerations being equal (15, 16); thus, while both have identical aerodynamic diameters, the large particles tend to exit the DPI more generally as single particles. The smaller particles aggregate more, leading to their deposition by gravity and inertia before reaching the "respirable" stages of the impactor.

To assess the influence of particle composition, we aerosolized a second type of porous particle (Fig. 1B), composed of poly(lactic acid-co-lysine-graft-lysine) (PLAL-Lys) (11). The PLAL-Lys particles exhibit some hygroscopicity, possibly a result of their lysine content, whereas the PLGA particles do not. Porous PLAL-Lys aerosols ($d = 8.2 \mu\text{m}$, $\rho < 0.1 \text{ g/cm}^3$) exhibited an in vitro respirable fraction ($57 \pm 1.9\%$) similar to that of the porous PLGA particles ($50 \pm 10\%$), which suggests that absolute particle mass density, rather than particle chemistry or hygroscopicity, is the prime determinant of the relatively high respirable fractions observed for the large porous particles. The values of $50 \pm 10\%$ and $57 \pm 1.9\%$ for porous particles exceed comparable respirable fractions obtained in recent aerosolization studies (15) performed with mannitol ($4 \pm 0.3\%$) and recombinant human granulocyte colony-stimulating factor (blended with mannitol) ($34 \pm 2\%$) powders using a Spinhaler DPI at a similar airflow rate (30 liter/min).

To determine whether the relatively efficient in vitro aerosolization of large porous particles translates into improved respirable fractions in vivo, we aerosolized porous and nonporous particles into the airways of rats

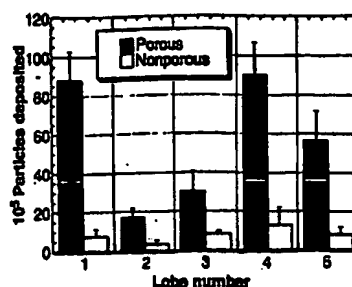


Fig. 2. Total particle recovery in rat lungs after bronchoalveolar lavage. Lobe numbers correspond to (1) left lung, (2) anterior, (3) median, (4) posterior, and (5) postcaval. For porous PLAL-Lys particles, $d = 8.9 \pm 4.2 \mu\text{m}$ and $\rho = 0.1 \text{ g/cm}^3$. For nonporous PLA particles, $d = 8.7 \pm 3.2 \mu\text{m}$ and $\rho = 0.94 \text{ g/cm}^3$. Means and SEs are based on $n = 4$.

(17). During forced ventilation, rats were exposed to porous or nonporous particles; bronchoalveolar lavage was used to collect particles deposited in the trachea as well as in the airways and acini (18). The nonporous particles deposited primarily in the trachea ($\sim 79\%$ of all particle mass that entered the trachea), whereas only 46% of the porous particle mass deposited in the trachea. Particles remaining in the rat lungs after bronchoalveolar lavage were obtained by careful dissection of the individual lobes of the lungs in subsequent experiments (19) (Fig. 2). The absolute number of porous particles remaining in the lungs was approximately an order of magnitude greater than the corresponding number of nonporous particles.

The role of low mass density in rendering large particles respirable can be understood in terms of the particles' mean aerodynamic diameter (12). Relatively large particles with high porosity have the same aerodynamic diameter as smaller, nonporous particles; these larger particles can enter the lungs because particle mass dictates the location of aerosol deposition in the lungs. The increased aerosolization efficiency of large, light particles lowers the probability of deposition losses before particle entry into the airways, thereby increasing the systemic bioavailability of an inhaled drug.

To test whether large particle size can increase systemic bioavailability, we encapsulated insulin into porous and nonporous polymeric particles. We designed the mass densities and mean diameters of the two particles such that they each had an aerodynamic diameter ($\sim 2 \mu\text{m}$) suitable for deep lung deposition (12); the mean diameters of the porous and nonporous particles were $> 5 \mu\text{m}$ and $< 5 \mu\text{m}$, respectively (Fig. 3, A to C). Identical masses of the porous or nonporous particles were administered to rats as an inhalation aerosol or injected subcutaneously (controls)

(20). Serum insulin concentrations were monitored as a function of time after inhalation or injection. For both porous (Fig. 3A) and nonporous (Fig. 3B) particles, blood levels of insulin reached high values within the first hour after inhalation. Only with large porous particles did blood levels of insulin remain elevated ($P < 0.05$) beyond 4 hours, with a relatively constant insulin release continuing to at least 96 hours ($0.04 < P < 0.2$). These results were confirmed by serum glucose values (Fig. 3C), which show falling glucose levels for the first 10 hours after inhalation of the porous insulin particles, followed by relatively constant low glucose levels for the remainder of the 96-hour period [for small nonporous insulin particles, initially suppressed glucose values rose after 24 hours (21)]. Similar biphasic release profiles of macromolecules from PLGA polymers have been

reported (22). For the large porous particles, insulin bioavailability relative to subcutaneous injection (23) was 87.5%, whereas the small nonporous particles yielded a relative bioavailability of 12% after inhalation. By comparison, bioavailability (relative to subcutaneous injection) of insulin administered to rats as an inhalation liquid aerosol has been reported as 37.3% using a similar endotracheal method (24). The absolute bioavailability of insulin inhaled into rat lungs in the form of a lactose-insulin powder through a DPI connected to an endotracheal tube has been reported as 6.5% (25). The longest sustained insulin release previously reported (6 hours) was achieved using liposomes intratracheally instilled into rat lungs (26).

Given the short systemic half-life of insulin (11 min) (27) and the 12- to 24-hour time scale of particle clearance from the central

and upper airways (5), the appearance of exogenous insulin in the bloodstream several days after inhalation appears to indicate that large porous particles achieve long, nonphagocytosed lifetimes in the deep lungs. To test this hypothesis, we lavaged (18) the lungs of rats immediately after inhalation of the porous and nonporous insulin particles as well as 48 hours after inhalation. For nonporous particles, $30 \pm 3\%$ of phagocytic cells contained particles immediately after inhalation, and $39 \pm 5\%$ contained particles 48 hours after inhalation. By contrast, only $8 \pm 2\%$ of phagocytic cells contained large porous particles immediately after inhalation, and $12.5 \pm 3.5\%$ contained particles 48 hours after inhalation. For small nonporous particles, $17.5 \pm 1.5\%$ of the phagocytic cell population contained three or more particles 48 hours after inhalation, compared with $4 \pm 1\%$ for large nonporous particles. Inflammatory response was also elevated with small nonporous particles; neutrophils represented $34 \pm 12\%$ of the phagocytic cell population 48 hours after inhalation of the small nonporous particles, compared with $8.5 \pm 3.5\%$ for large porous particles (alveolar macrophages represented 100% of cells measured immediately after inhalation). These results are consistent with those of *in vitro* experiments showing that phagocytosis of particles diminishes precipitously as particle diameter increases beyond $3 \mu\text{m}$ (28).

To further determine whether increased bioavailability correlates with increasing size of porous particles, we encapsulated a second model drug, testosterone, in porous particles of two different mean geometric diameters (10.1 and $20.4 \mu\text{m}$). An identical mass of powder was administered to rats as an inhalation aerosol or as a subcutaneous injection (controls). Serum testosterone concentrations were monitored as a function of time after inhalation or injection (Fig. 3, D and E). Blood levels of testosterone remained well above background levels ($P < 0.05$) for 12 to 24 hours, even though the systemic half-life of testosterone is 10 to 20 min (27). Testosterone bioavailability relative to subcutaneous injection was 177% for the $20.4\text{-}\mu\text{m}$ -diameter particles (Fig. 3D) and 53% for the $10.1\text{-}\mu\text{m}$ -diameter particles (Fig. 3E). The increase in testosterone bioavailability with increasing size of porous particles is especially notable given that the mean diameter of the $20.4\text{-}\mu\text{m}$ particles is ~ 10 times that of nonporous conventional therapeutic particles (5, 10). The relatively short time scale of testosterone release observed for both the inhalation and subcutaneous controls is near the *in vitro* time scale of release (several hours) reported elsewhere for 50:50 PLGA microparticles of similar size encapsulating a therapeutic substance (bupivacaine) of similar molecular weight and lipophilicity (29).

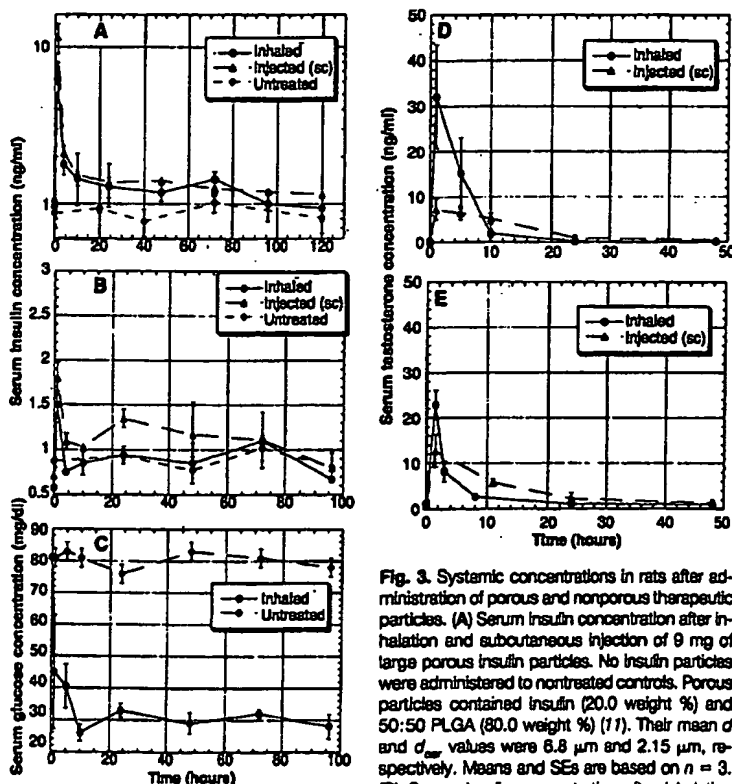


Fig. 3. Systemic concentrations in rats after administration of porous and nonporous therapeutic particles. (A) Serum insulin concentration after inhalation and subcutaneous injection of 9 mg of large porous insulin particles. No insulin particles were administered to nontreated controls. Porous particles contained insulin (20.0 weight %) and 50:50 PLGA (80.0 weight %) (11). Their mean d and d_{50} values were $6.8 \mu\text{m}$ and $2.15 \mu\text{m}$, respectively. Means and SEs are based on $n = 3$. (B) Serum insulin concentration after inhalation

and subcutaneous injection of 9 mg of small nonporous insulin particles. No insulin particles were administered to nontreated controls. Nonporous particles contained insulin (10.0 weight %) and 50:50 PLGA (90.0 weight %) (11). Their mean d and d_{50} values were $4.4 \mu\text{m}$ and $2.15 \mu\text{m}$, respectively. Means and SEs are based on $n = 3$. (C) Serum glucose concentration after inhalation of 9 mg of large porous insulin particles or 9 mg of small nonporous insulin particles. No insulin particles were administered to nontreated controls. Means and SEs are based on $n = 3$. (D) Serum testosterone concentration after administration of 8 mg of porous testosterone particles ($d = 20.4 \mu\text{m}$) as an inhalation powder and as a subcutaneous control. Particles contained testosterone (15 weight %), 50:50 PLGA (78.5 weight %), and PLAL-Lys (8.5 weight %). For the dry powder, $\rho = 0.1 \text{ g/cm}^3$. (E) Same as (D) but with smaller porous testosterone particles ($d = 10.1 \mu\text{m}$).

Porous particles comprising therapeutics and pharmaceutical excipients can easily be formed by spray-drying (30), rapid expansion of supercritical fluids (31), and other particle formation technologies. Hence, they can immediately address a variety of needs as therapeutic carriers for inhalation therapies. Their potential for high aerosolization efficiency, long-term drug release, and increased systemic bioavailability makes large porous particles especially attractive for systemic inhalation therapies.

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12. The aerodynamic diameter d_{ae} is related to the actual sphere diameter d by the formula

$$d_{ae} = d \sqrt{\rho} \quad (1)$$
 (5). Maximal deposition of monodisperse aerosol particles in the alveolar region of the human lung (~60% of the inhaled particles) occurs for $d_{ae} = \sim 3 \mu$ m (32). Inhaled particles with $d_{ae} > 3 \mu$ m tend to deposit in the upper airways by gravity and inertia, whereas particles with $d_{ae} < 3 \mu$ m tend to be exhaled. On the basis of Eq. 1, the actual diameter d (in micrometers) of porous particles that will exhibit maximum deep-lung deposition because of their small ρ is

$$d = 3/\sqrt{\rho} \quad (2)$$
 (where $\rho < 1$). Hence, for such particles d is always greater than 3 μ m. For example, porous particles with $d = 8.5 \mu$ m and $\rho = 0.1$ g/cm³ have approximately the same aerodynamic diameter (~2.7 μ m) as nonporous particles with $d = 3.5 \mu$ m and $\rho = 0.8$ g/cm³.
13. We placed 20 mg of porous or nonporous microparticles, with encapsulated rhodamine as a fluorescent marker, in No. 1 hard gelatin capsules (El Lys, loaded the capsules into a Spinhater DPI (Fisons, Bedford, MA), and activated the DPI. Particles were aerosolized into a Mark I Andersen Impactor (Andersen Samplers, Atlanta, GA) from the Spinhater device for 30 s at a flow rate of 28.3 liter/min. [Each plate of the Impactor was previously coated with Tween 80 by immersing the plates in an acetone solution (5% w/v) and then evaporating the acetone in an oven at 60°C for 5 min.] After aerosolization and deposition, particles were collected from each stage of the impactor system and completely degraded in NaOH solution (0.2 N). After incubation at 37°C for 12 hours, the fluorescence of each solution was measured (wavelengths of 554 nm excitation, 574 nm emission).
14. Particle mass density was determined either by non-mercury porosimetry or tap density measurements (Micromeritics, Norcross, GA). The mass density of nonporous particles ranged from ~0.4 to 1.0 g/cm³. The lower mass density limit (0.4 g/cm³) is near that recently reported for spray-dried pharmaceutical powders (15), whereas the upper limit (1.0 g/cm³) is similar to the mass density of liquid pharmaceutical aerosols (A. Adjei et al., *Int. J. Pharm.* 107, 57 (1994)) and other dry-powder aerosols (M. T. Vidgran, P. A. Vidgran, T. P. Peronen, *ibid.* 35, 139 (1987)). The mass density of porous particles was typically near or equal to 0.1 g/cm³. Mass density variation from particle to particle within powders did not appear to introduce a substantial source of uncertainty. For example, using an API aerosolizer, we measured the mean time-of-flight of the porous and nonporous particles described in the legend of Fig. 2. This gave a mean d_{ae} of $1.57 \pm 2.41 \mu$ m for the porous particles and $5.82 \pm 1.88 \mu$ m for the nonporous particles. Using Eq. 1 in (12), this indirectly yields a mass density ratio (porous to nonporous) of 0.1 ± 0.3 . Given that the measured ratio of mass densities is 0.11 (Fig. 2), mass density variation within the two powders appears to be small (<10%).
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17. Male Sprague-Dawley rats (150 to 200 g) were anesthetized using ketamine (80 mg/kg)-xylazine (10 mg/kg). Anesthetized rats were placed ventral side up on a surgical table provided with a temperature-controlled pad to maintain physiological temperature. Rats were cannulated above the carina with an endotracheal tube connected to a Harvard ventilator and were force-ventilated for 20 min at 300 ml/min. Fifty micrograms of porous (PLAL-Lys) or nonporous (PLA) microparticles were introduced into the endotracheal tube. After the period of forced ventilation, rats were killed and the lungs and trachea were separately washed using bronchoalveolar lavage (18). The lavage fluid was centrifuged (400g), and the pellets were collected and resuspended in 2 ml of phenol red-free Hank's balanced salt solution (HBSS) (Gibco) without Ca²⁺ and Mg²⁺. A 100- μ l sample was removed for particle counting using a hemacytometer. The remaining solution was mixed with 10 ml of 0.4 M NaOH. After incubation at 37°C for 12 hours, the fluorescence of each solution was measured (wavelengths of 554 nm excitation, 574 nm emission).
18. Lungs were lavaged immediately (5 to 10 min) after inhalation. A tracheal cannula was inserted and tied into place, and the airways were washed with 10-ml aliquots of HBSS. The lavage procedure was repeated until a total volume of 30 ml was collected. In the phagocytic cell experiments, lavage fluid was centrifuged and the cell pellets were resuspended in HBSS for counting, differentiation of cell types, and measurement of phagocytosis. Engulfment of particles by phagocytic cells was determined by counting microscopically the number of particles incorporated per cell in wet mounts and in fixed and stained cytocentrifuge preparations. Reported numbers are based on the wet-mount experiments; similar numbers were obtained using the stained cytocentrifuge preparations.
19. The lobes were placed in separate petri dishes containing 5 ml of HBSS. Each lobe was teased through a 60-mesh screen to dissociate the tissue and was then filtered through cotton gauze to remove tissue debris and connective tissue. The petri dish and gauze were washed with an additional 15 ml of HBSS to maximize microparticle collection. Each tissue preparation was centrifuged and resuspended in 2 ml of HBSS, and the particles were counted in a hemacytometer.
20. Rats were anesthetized and cannulated as described (17) and were force-ventilated for 10 to 20 min at 300 ml/min. Rats received two types of aerosols through the endotracheal tube. After the period of forced ventilation, necks were sutured and rats were reawakened within 1 to 2 hours. Blood samples (300 μ l) were periodically withdrawn from the tail vein over a period of 2 to 6 days. These samples were mixed with assay buffer, centrifuged, and the supernatant examined for the presence of (endogenous and exogenous) insulin or testosterone using radioimmunoassays (ICN Pharmaceuticals, Costa Mesa, CA). Glucose was measured using a colorimetric assay (Sigma). Control studies involved subcutaneous injection of the same amount of powder as was inhaled. The particles were injected into the scruff of the neck.
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33. We thank J. Heidel and C. Perez de la Cruz for helpful technical assistance. Supported by an NSF CAREER Grant (D.E., J.M.) and NIH grants GM26698 (R.L.) and HD29125 (R.L., J.H.).

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Acta Cryst. (1965). 19, 479

Crystal data (II) for some androstanes¹. By JEAN M. ORT, BARBARA A. HANER and DONITA A. NORTON, *Biophysics Department, Roswell Park Memorial Institute, Buffalo, New York 14203, U.S.A.*

(Received 2 February 1965)

Norton, Lu & Campbell (1962) reported the single-crystal data for a series of androstanes. This paper reports the same data for a second set of androstanes (Table 1) using the

same techniques described in the earlier paper. No further work on these compounds is contemplated.

Reference

* This investigation was supported in part by Public Health Service Research Grant CA-06183 from the National Cancer Institute.

NORTON, D. A., LU, C. T. & CAMPBELL, A. E. (1962). *Acta Cryst.* 15, 1189.

Table 1. Crystal data (II) for some androstanes

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|--------------------------------------|--|--|--|--|--|--|--|--|--|
| Formula | C ₁₉ H ₂₈ O ₂ | C ₁₉ H ₂₈ O ₂ | C ₁₉ H ₂₈ O ₂ | C ₁₉ H ₂₈ O ₂ | C ₁₉ H ₂₈ O ₂ | C ₁₉ H ₂₈ O ₂ | C ₁₉ H ₂₈ O ₂ | C ₂₁ H ₃₂ O ₃ | C ₂₂ H ₃₄ O ₃ |
| Mol. Wt. | 286.40 | 286.40 | 288.41 | 288.41 | 290.43 | 290.43 | 300.38 | 330.45 | 394.53 |
| D _m (g.cm ⁻³) | 1.178 | 1.164 | 1.147 | 1.186 | 1.132 | 1.147 | 1.264 | 1.162 | 1.148 |
| D _x (g.cm ⁻³) | 1.175 | 1.174 | 1.143 | 1.177 | 1.103 | 1.084 | 1.253 | 1.201 | 1.207 |
| Space group | P2 ₁ 2 ₁ 2 ₁ | P2 ₁ 2 ₁ 2 | A2 | P2 ₁ | P2 ₁ | P2 ₁ 2 ₁ 2 ₁ | P2 ₁ 2 ₁ 2 ₁ | P2 ₁ 2 ₁ 2 ₁ | P2 ₁ |
| Z | 4 | 4 | 4 | 4 | 2 | 4 | 4 | 4 | 2 |
| a (Å)* | 12.963 | 12.302 | 21.337 | 14.691 | 11.614 | 12.146 | 9.263 | 12.800 | 10.860 |
| b (Å)* | 16.929 | 18.644 | 6.186 | 11.093 | 8.096 | 23.434 | 26.531 | 18.169 | 16.241 |
| c (Å)* | 7.366 | 7.065 | 12.704 | 10.872 | 9.422 | 6.248 | 6.477 | 7.856 | 6.236 |
| β (°) | — | — | 91.27 | 113.23 | 99.23 | — | — | — | 99.17 |
| V (Å ³) | 1619 | 1620 | 1676 | 1628 | 874 | 1779 | 1592 | 1627 | 1086 |
| Solvent | Ethanol | Methanol | Unknown | Methanol | Ethanol | Ethanol | Methanol—acetone | Methanol | Ethanol |

* ± 0.009 Å

1. 4-Androsten-3,17-dione (androstenedione)
2. 1,4-Androstadien-17β-ol-3-one (1-dehydrotestosterone)
3. 5α-Androstan-3,17-dione (androstanedione)
4. 4-Androsten-17β-ol-3-one (testosterone)
5. 5α-Androstan-17β-ol-3-one (alldihydrotestosterone)
6. 5-Androsten-3β, 17β-diol (adrenostenediol)
7. 4-Androsten-3,11,17-trione (adrenosterone)
8. 4-Androsten-17β-ol-3-one 17-acetate (testosterone acetate)
9. 5α-Androstan-17β-ol-3-one 17-benzoate (dihydrotestosterone benzoate)

Acta Cryst. (1965). 19, 479

An X-ray investigation of the stereochemistry of Zn(NCS)₂(C₆H₅NH₂)₂. By T. M. SHEPHERD and IDA WOODWARD, *Chemistry Department, Queen's University of Belfast, Belfast, Northern Ireland.*

(Received 22 June 1964)

The stereochemistry of the complex Zn^{II}(NCS)₂(C₆H₅NH₂)₂ is of interest in connection with studies in these laboratories on the factors governing the configuration of metal complexes. Nelson & Shepherd (unpublished work) have established, from magnetic and spectral data, that the corresponding Co(II) and Ni(II) complexes have octahedral (or tetragonal) structures with bridging NCS groups. X-ray powder patterns show that these and the corresponding Cd(II) complex are isomorphous, but that Zn(NCS)₂(C₆H₅NH₂)₂ has a different crystal structure. An attempt to determine this structure sufficiently to establish the coordination number of the zinc atom is described here.

Single crystals obtained by recrystallization from ethanol were used. These were needle-shaped, approx 1.5 mm long,

and 0.3 mm in cross section. The needle axis being taken as the c axis, rotation photographs with Cu Kα radiation about the [001] and [110] axes and Laue photographs showed the unit cell to be orthorhombic with the cell dimensions:

$$a = 14.56 \pm 0.05 \text{ Å}$$

$$b = 9.10 \pm 0.05$$

$$c = 12.7 \pm 0.1$$

The values of a and b were derived from the observed spacings of 39 hkl reflexions, and their uncertainties are an estimate based on the standard deviations of the observed and calculated values. c was obtained from layer line measurements of a rotation photograph.

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Acta Cryst. (1970). B26, 1184

Crystallographic data for testosterone hydrate and anhydrate. By A. L. THAKKAR, N. D. JONES, H. A. ROSE, L. G. TENSMAYER and N. A. HALL,* *Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46206, U.S.A.*

(Received 15 January 1970)

Testosterone hydrate crystallizes in the space group $P2_12_12_1$ with four molecules in a unit cell having the dimensions $a=13.63$, $b=15.95$ and $c=7.94$ Å. Anhydrous testosterone crystallizes in the space group $P2_1$ with four molecules in the unit cell. The proper cell dimensions are $a=14.45$, $b=11.09$, $c=10.88$ Å and $\beta=110.5^\circ$.

In a previous study on the solution behavior of testosterone in aqueous media, conversion of the anhydrate form to a hydrate was reported (Thakkar & Hall, 1969). Since testosterone is a natural hormone and exists in an aqueous environment, characterization of this form is important. We wish to report here the crystallographic parameters of the hydrate.

Small single-crystals were grown by a continuous fall method from saturated aqueous solution cooled from 33.0 to 29.5°C at 0.1°C per hour. Elemental analysis, Karl Fischer titration and thermogravimetric analysis showed this crystalline form to be the monohydrate.

From Weissenberg and precession photographs taken with Cu K α radiation the space group has been found to be $P2_12_12_1$ (systematic absences: $h00$, $0k0$, and $00l$ for h , k or l odd); there are four molecules in a unit-cell having the dimensions $a=13.63$, $b=15.95$ and $c=7.94$ Å. The density measured by displacement is 1.181 g.cm $^{-3}$, which agrees well with the calculated density for C $_{19}$ H $_{28}$ O $_2$ ·H $_2$ O of 1.179 g.cm $^{-3}$.

* Present address: College of Pharmacy, University of Washington, Seattle, Washington 98105.

Acta Cryst. (1970). B26, 1184

Crystal data of BaSrFe $_4$ O $_{11}$. By S. MERIANI and G. SLOCCARI, *Istituto di Chimica Applicata dell'Università di Trieste, via Valerio 2, Trieste, Italy.*

(Received 20 January 1970)

The dimensions of the orthorhombic unit cell of BaSrFe $_4$ O $_{11}$, which contains two formula units, are $a=5.516$, $b=8.265$, $c=9.188$ Å. The space group is $Pna2_1$.

A previous report on the phase equilibrium diagram, BaO-SrO-Fe $_2$ O $_3$, shows that a new stable compound, having the composition BaSrFe $_4$ O $_{11}$, may occur as a single phase above 1100±10°C (Batti, 1962). It undergoes thermal transformation at about 1200°C and melts incongruently at 1240±10°C. A further investigation by Barbariol & Batti (1968) established that this new phase forms a solid solution with the binary compound BaFe $_2$ O $_7$, which is reported to be orthorhombic (Okazaki, Mori & Mitsuda,

1963; DoDinh & Bertaut, 1965). They display complete solubility above 1200°C whereas at lower temperatures a solid-solution gap of increasing width was reported.

Single crystals of BaSrFe $_4$ O $_{11}$ were grown by solid-state reaction, from a pressed pellet mixture of 1 BaCO $_3$:1 SrCO $_3$:2 Fe $_2$ O $_3$, which was heated on a platinum strip in a resistance furnace to about 950°C. The sintered pellet was reground and refired to assure complete reaction. The microcrystalline specimen was brought to 1200°C and left in the furnace

For comparison we have measured the crystal parameters for anhydrous testosterone and have obtained values which differ from those reported by Bernal & Crowfoot (1936). The space group is $P2_1$ with four molecules in a unit-cell having the dimensions $a=14.73$, $b=11.09$, $c=10.88$ Å and $\beta=113.3^\circ$, which agree fairly well with the values given by Ohrt, Haner & Norton (1965). There is, however, an alternative cell with β closer to 90°. The dimensions for this proper cell are $a=14.45$, $b=11.09$, $c=10.88$ Å and $\beta=110.5^\circ$. These cells give a calculated density of 1.173 g.cm $^{-3}$, which is identical with the experimentally measured value given by Bernal & Crowfoot.

The indexed powder data for these two forms of testosterone will be submitted for inclusion in the *ASTM Powder Diffraction File*.

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Acta Cryst. (1964). 17, 1611

Crystal data (II) for some estrone-related compounds.* By JEAN M. OHRT, BARBARA A. HANER, and DORRIS A. NORTON, *Department of Biophysics, Roswell Park Memorial Institute, Buffalo, New York, U.S.A.*

(Received 1 May 1964)

The lattice constants of eleven estrone-related compounds (Table I) were determined on a General Electric XRD 5 X-ray diffraction unit equipped with a goniostat using $\text{Cu K}\alpha$ radiation. Space groups were established on the basis of systematic absences and optical activity.

All crystals were grown from solution. Flotation density measurements were used to calculate the number of molecules per unit cell. The high discrepancy between the measured (D_m) and calculated (D_c) densities of compound (3) is due to the crystallization of one molecule of ethanol per molecule of steroid. The density calculated taking the solvent of crystallization molecules into

consideration is 1.151 g.cm^{-3} which agrees with the measured density. All of the other measured and calculated densities also agree within the experimental error (4.0%).

Compounds (1) and (2) each crystallized in two forms. Both of the (a) forms had similar but non-isomorphous unit cells. Compounds (2) and (3) are isomers but do not form isomorphous crystals.

* This investigation was supported in part by P.H.S. grant CY-06183 from the National Cancer Institute, Public Health Service.

Table I. Crystal data (II) for some estrone-related compounds

- (1)(a), (b) 1,3,5(10)-Estratrien-3-ol-17-one
(2)(a), (b) 1,3,5(10)-Estratrien-3,17 α -diol
(3) 1,3,5(10)-Estratrien-3,17 β -diol
(4) 1,3,5(10)-Estratrien-3,17 β -diol 3-methyl ether
(5) 1,3,5(10)-Estratrien-3,17 β -diol 17-acetate
(6) 1,3,5(10)-Estratrien-17 α -ethinyl-3,17 β -diol 3-methyl ether
(7) 1,3,5(10)-Estratrien-3,17 α -diol diacetate
(8) 1,3,5(10)-Estratrien-3-ol-17-one trimethyl acetate
(9) 1,3,5(10)-Estratrien-3,17 α -diol 3-trimethyl acetate
(10) 1,3,5(10)-Estratrien-3,17 β -diol dipropionate
(11) 1,3,5(10)-Estratrien-3,17 β -diol 3-benzoate

| | 1(a) | 1(b) | 2(a) | 2(b) | 3 | 4 | 5 |
|------------------------------|--|--|--|--|--|--|--|
| Formula | $\text{C}_{18}\text{H}_{26}\text{O}_2$ | $\text{C}_{18}\text{H}_{26}\text{O}_2$ | $\text{C}_{18}\text{H}_{26}\text{O}_2$ | $\text{C}_{18}\text{H}_{26}\text{O}_2$ | $\text{C}_{18}\text{H}_{26}\text{O}_2$ | $\text{C}_{18}\text{H}_{26}\text{O}_2$ | $\text{C}_{18}\text{H}_{26}\text{O}_2$ |
| Mol. wt. | 270.37 | 270.37 | 272.39 | 272.39 | 272.39 | 286.42 | 314.43 |
| D_m (g.cm^{-3}) | 1.17 ₆ | 1.24 ₆ | 1.20 ₁ | 1.20 ₁ | 1.14 ₆ | 1.20 ₁ | 1.20 ₁ |
| D_c (g.cm^{-3}) | 1.220 | 1.249 | 1.190 | 1.190 | 0.985 | 1.198 | 1.220 |
| Space group | $P2_1$ | $P2_12_12_1$ | $P2_1$ | $C2$ | $P2_12_12_1$ | $P2_12_12_1$ | $P2_12_12_1$ |
| Z (calc.) | 4 | 4 | 4 | 4 | 4 | 8 | 4 |
| a (Å)† | 9.297 | 10.051 | 9.127 | 10.212 | 12.241 | 14.004 | 13.803 |
| b (Å)† | 23.317 | 18.440 | 23.293 | 7.132 | 23.215 | 35.458 | 16.912 |
| c (Å)† | 7.682 | 7.755 | 7.238 | 13.422 | 6.466 | 6.470 | 7.331 |
| β (°) | 113.26 | — | 99.78 | 124.25 | — | — | — |
| Volume (Å ³) | 1471 | 1437 | 1621 | 1620 | 1837 | 3213 | 1711 |
| Solvent | Methanol | Acetone | Methanol | Methanol | 95% Ethanol | Heptane | 95% Ethanol |

† ± 0.007 .

Table I (cont.)

| | 6 | 7 | 8 | 9 | 10 | 11 |
|------------------------------|--|--|--|--|--|--|
| Formula | $\text{C}_{18}\text{H}_{26}\text{O}_2$ | $\text{C}_{18}\text{H}_{26}\text{O}_2$ | $\text{C}_{18}\text{H}_{26}\text{O}_2$ | $\text{C}_{18}\text{H}_{26}\text{O}_2$ | $\text{C}_{18}\text{H}_{26}\text{O}_2$ | $\text{C}_{18}\text{H}_{26}\text{O}_2$ |
| Mol. wt. | 310.44 | 356.47 | 354.49 | 356.51 | 384.52 | 376.50 |
| D_m (g.cm^{-3}) | 1.22 ₁ | 1.20 ₁ | 1.16 ₁ | 1.14 ₁ | 1.20 ₁ | 1.21 ₁ |
| D_c (g.cm^{-3}) | 1.317 | 1.246 | 1.175 | 1.153 | 1.182 | 1.251 |
| Space group | $P2_1$ | $P2_12_12_1$ | $P2_1$ | $P2_1$ | $P2_1$ | $P2_12_12_1$ |
| Z (calc.) | 4 | 4 | 2 | 2 | 4 | 4 |
| a (Å)† | 6.998 | 8.663 | 20.704 | 16.805 | 23.038 | 13.331 |
| b (Å)† | 39.737 | 29.390 | 7.318 | 9.714 | 9.057 | 23.848 |
| c (Å)† | 6.871 | 7.460 | 6.781 | 6.356 | 10.352 | 6.286 |
| β (°) | 117.58 | — | 102.61 | 98.35 | 95.36 | — |
| Volume (Å ³) | 1694 | 1899 | 1003 | 1027 | 2160 | 1998 |
| Solvent | Heptane | Methanol | Propanol | Heptane-acetone | Acetone-methanol | 95% Ethanol |

† ± 0.007 .

ACTA CRYSTALLOGRAPHICA



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DL-N-1-BUTYL-2(4-HYDROXY-3-HYDROXYMETHYLPHENYL)2-HYDROXYETHYLAMINE, (SAL-
BUTAMOL, Ah.3365), $C_{13}H_{21}NO_3$

by J.P.Besle* and C.T.Grainger
Crystallography Department, The University of New South Wales, Box 1, P.O.
Kensington, N.S.W. 2033, Australia

Introduction. The crystal structure of Salbutamol has been determined by X-ray diffraction. Salbutamol is one compound of a new series of β -adrenergic stimulants which have been found to have considerably greater action on bronchial smooth muscle than on other smooth muscle affected by β -stimulants, and is currently being used in many countries for the treatment and management of asthma (Hartley, Jack, Lunts and Ritchie, 1968; Brittain, Farmer, Jack, Martin and Simpson, 1968).

Crystal Data. Rectangular crystals were obtained by recrystallisation from water. The space group as determined from systematic spectral absences is $Pbca$; the unit cell dimensions are: $a = 21.654(10)$, $b = 8.798(4)$, $c = 14.565(7)$ Å; $Z = 8$; $\rho_{obs} = 1.15 \text{ g cm}^{-3}$, $\rho_{calc} = 1.15 \text{ g cm}^{-3}$.

Data Collection, Structure Elucidation and Refinement. A total of 2644 independent reflections were measured on a Siemens automatic single crystal diffractometer using $\text{CuK}\alpha$ radiation and nickel attenuators (Craig, 1971). The structure was solved using direct phasing procedures incorporating programmes NORM, PHREL and GSAM written by Grainger. Reflections having $|E_{obs}| \geq 1.60$ (272 in number) were selected and used in the phase analysis and calculation of an E map from which the positions of all non-hydrogen atoms were obtained. Eventually all atoms (including hydrogens) were located and the structure refined by full matrix least squares procedures to an overall discrepancy (R) factor of 0.048. It is worth noting that comparison between the fully refined phases of the initial 272 selected reflections and those used to phase the E map revealed that only one phase was incorrectly assigned in the first instance.

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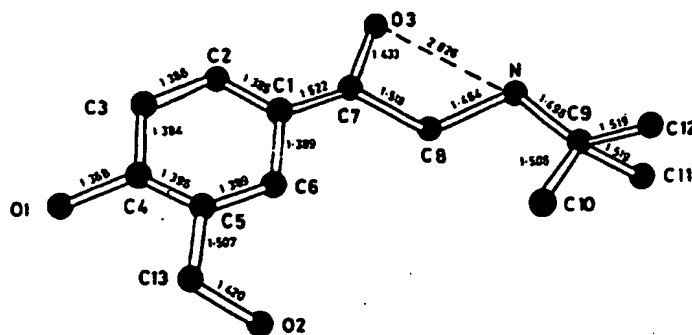


Fig.1. A diagram of a molecule of Salbutamol showing some intramolecular bond distances.



Fig. 2.

| <u>Comment</u> |
|----------------|
| xyl gro |
| (2.826(|
| (2.766(|

Bond Distances and Angles

ⁱ⁾ \pm/σ

- 1) 0.169(2)
- 1) 0.373(2)
- 1) 0.390(2)
- 1) 0.519(2)
- 1) 0.501(2)
- 1) 0.445(2)
- 1) 0.536(2)
- 1) 0.535(2)
- 1) 0.453(2)
- 1) 0.620(2)
- 1) 0.650(2)
- 1) 0.710(2)
- 1) 0.729(2)
- 1) 0.619(2)
- 1) 0.679(2)
- 1) 0.707(2)
- 1) 0.503(2)
- 1) 0.552(2)
- 1) 0.473(2)

| | | | |
|-----------------|------------|------------------|------------|
| O(1)-C(4) | 1.368(2) Å | C(6)-C(1) | 1.389(3) Å |
| O(2)-C(13) | 1.420(2) | C(1)-C(7) | 1.521(2) |
| O(3)-C(7) | 1.433(2) | C(7)-C(8) | 1.518(3) |
| C(1)-C(2) | 1.385(3) | C(8)-N | 1.464(2) |
| C(2)-C(3) | 1.386(3) | N-C(9) | 1.496(2) |
| C(3)-C(4) | 1.384(3) | C(9)-C(10) | 1.506(4) |
| C(4)-C(5) | 1.396(3) | C(9)-C(11) | 1.519(3) |
| C(5)-C(13) | 1.507(3) | C(9)-C(12) | 1.519(4) |
| C(5)-C(6) | 1.389(2) | | |
| O(1)-C(4)-C(3) | 123.2(2)° | C(6)-C(1)-C(2) | 115.9(2)° |
| O(1)-C(4)-C(5) | 116.2(2) | C(6)-C(1)-C(7) | 120.4(2) |
| O(2)-C(13)-C(5) | 110.2(2) | C(2)-C(1)-C(7) | 120.7(2) |
| C(13)-C(5)-C(4) | 119.0(2) | C(1)-C(7)-C(8) | 110.8(1) |
| C(13)-C(5)-C(6) | 122.6(2) | C(7)-C(8)-N | 111.5(2) |
| O(3)-C(7)-C(1) | 111.5(1) | C(8)-N-C(9) | 115.9(1) |
| O(3)-C(7)-C(8) | 107.0(2) | N-C(9)-C(10) | 105.6(2) |
| C(1)-C(2)-C(3) | 120.6(2) | N-C(9)-C(11) | 112.9(2) |
| C(2)-C(3)-C(4) | 119.9(2) | N-C(9)-C(12) | 105.6(1) |
| C(3)-C(4)-C(5) | 120.6(2) | C(10)-C(9)-C(11) | 109.3(2) |
| C(4)-C(5)-C(6) | 118.3(2) | C(10)-C(9)-C(12) | 110.7(3) |
| C(5)-C(6)-C(1) | 121.6(2) | C(11)-C(9)-C(12) | 109.7(2) |

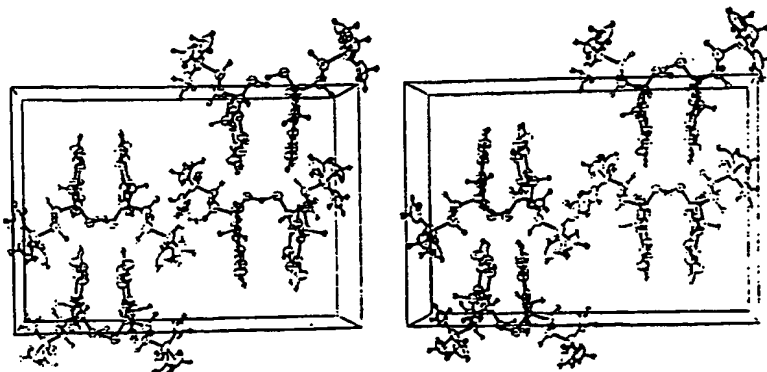


Fig.2. A packing diagram showing the unit cell contents of Salbutamol.

intramole-

Comments. Salbutamol exhibits the same cis orientation of amino and hydroxyl groups as observed with Th1165 (Seale, 1972). The N...O distance (2.826(2) Å) is in fact a little larger than the corresponding distance (2.766(3) Å) in Th1165. The benzene ring is inclined at 74.5(2)° to the

plane of the C(7)-C(8)-N-C(9) atoms, whilst in the Th1165 molecule this angle is $66.9(3)^\circ$. The tertiary butyl group is on the opposite side of the Salbutamol molecule to the amino and hydroxyl groups. The Th1165 molecule has the substituent groups on the nitrogen atom on the same side of the molecule as the amino and hydroxyl groups and therefore in closer proximity to the receptor site.

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 P. Main, M.M. Woolfson and G. Germain, "LSAM - A System of Computer Programs for the Automatic Solution of Centrosymmetric Crystal Structures", Private Communication (1971).

Received: 4 January 1972

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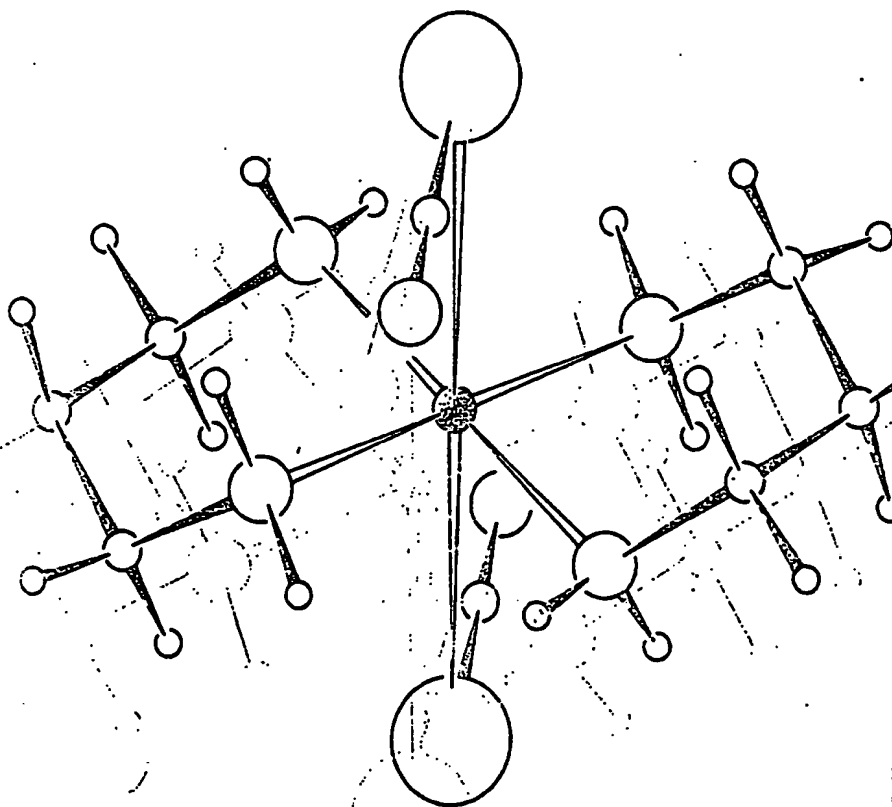
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